Devi, S.
09/412558

-key terms

FILE 'REGISTRY' ENTERED AT 14:31:37 ON 13 NOV 2001
E GONADOTROPIN RELEASING HORMONE/CN 5
L1 7 S GONADOTROPIN RELEASING HORMONE ?/CN

L6 1 S 9034-40-6/RN

L7 63 S "GONADOTROPIN-RELEASING HORMONE" ?/CN

FILE 'CAPLUS' ENTERED AT 14:48:58 ON 13 NOV 2001
L1 7 SEA FILE=REGISTRY ABB=ON PLU=ON GONADOTROPIN RELEASING HORMONE ?/CN
L6 1 SEA FILE=REGISTRY ABB=ON PLU=ON 9034-40-6/RN
L7 63 SEA FILE=REGISTRY ABB=ON PLU=ON "GONADOTROPIN-RELEASING HORMONE" ?/CN
L9 746 SEA FILE=CAPLUS ABB=ON PLU=ON PSEUDOMONAS(S)((EXOTOXIN OR EXO TOXIN)(W)A)
L10 501 SEA FILE=CAPLUS ABB=ON PLU=ON L9 AND (L1 OR L7 OR L6 OR PEPTIDE OR PROTEIN OR POLYPROTEIN OR POLYPEPTIDE OR GNRH OR (GN OR GONADOTROPIN)(W)(RH OR RELEAS? HORMON?) OR VACCINIA)
L11 12 SEA FILE=CAPLUS ABB=ON PLU=ON L10 AND RECEPTOR BIND? DOMAIN

L1 7 SEA FILE=REGISTRY ABB=ON PLU=ON GONADOTROPIN RELEASING HORMONE ?/CN
L6 1 SEA FILE=REGISTRY ABB=ON PLU=ON 9034-40-6/RN
L7 63 SEA FILE=REGISTRY ABB=ON PLU=ON "GONADOTROPIN-RELEASING HORMONE" ?/CN
L9 746 SEA FILE=CAPLUS ABB=ON PLU=ON PSEUDOMONAS(S)((EXOTOXIN OR EXO TOXIN)(W)A)
L10 501 SEA FILE=CAPLUS ABB=ON PLU=ON L9 AND (L1 OR L7 OR L6 OR PEPTIDE OR PROTEIN OR POLYPROTEIN OR POLYPEPTIDE OR GNRH OR (GN OR GONADOTROPIN)(W)(RH OR RELEAS? HORMON?) OR VACCINIA)
L12 9 SEA FILE=CAPLUS ABB=ON PLU=ON L10 AND (REPETIT? OR REPEAT?)

L13 19 L11 OR L12

L13 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:334105 CAPLUS
TITLE: A recombinant chimera composed of **repeat** region RR1 of Mycoplasma hyopneumoniae adhesin with Pseudomonas exotoxin: in vivo evaluation of specific IgG response in mice and pigs
AUTHOR(S): Chen, J.-R.; Liao, C.-W.; Mao, S. J. T.; Weng, C.-N.
CORPORATE SOURCE: Department of Pathobiology, Pig Research Institute Taiwan, Chunan Miaoli, 35099, Taiwan
SOURCE: Vet. Microbiol. (2001), 80(4), 347-357
CODEN: VMICDQ; ISSN: 0378-1135
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Using the binding and translocation domain of **Pseudomonas exotoxin A** [domain III deleted PE termed

PE(.DELTA.III)] as a vehicle, this study characterized and evaluated a novel application of PE toxin in Mycoplasma hyopneumoniae adhesin used as an immunogen. PCR and sequence anal. revealed that 16 copies of AAKPV(E) in tandem **repeat** region 1 (RR1) of M. hyopneumoniae 97 kDa adhesion were successfully fused to the downstream of PE(.DELTA.III) to create a subunit vaccine, i.e. PE(.DELTA.III)-RR1. This chimeric **protein**, over-expressed in inclusion bodies of E. coli BL21(DE3)pLysS, was characterized by a monoclonal antibody (MAb) F2G5 prepd. against RR1 of the 97 kDa adhesin and was readily purified. The data indicated that the epitope recognized by MAb F2G5 was located in the structure of PE(.DELTA.III)-RR1. Using ELISA and Western blot analyses, the specific IgG immune response against RR1 and whole adhesin in mice immunized with PE(.DELTA.III)-RR1 was found more marked than that in mice immunized with the M. hyopneumoniae whole cells. Similarly, PE(.DELTA.III)-RR1 also stimulated a remarkable IgG response against RR1 in pigs compared to that in pigs immunized with the conventional M. hyopneumoniae vaccine. The PE(.DELTA.III)-RR1 would be potentially useful for the future development of a M. hyopneumoniae adhesin vaccine.

REFERENCE COUNT: 26

REFERENCE(S):
(1) Ashcom, J; J Cell Biol 1990, V110, P1041 CAPLUS
(2) Bagdasarian, M; Vaccine 1999, V17, P441 CAPLUS
(3) Chen, J; Vet Microbiol 1998, V62, P97 CAPLUS
(4) Eidels, L; Microbiol Rev 1983, V47, P596 CAPLUS
(5) FitzGerald, D; J Biol Chem 1998, V273, P9951 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:261138 CAPLUS
DOCUMENT NUMBER: 134:294520
TITLE: Method for making fusion **protein** vaccines using **repeat** immunogens and **receptor binding domain** of a Pseudomonas exotoxin
INVENTOR(S): Hwang, Jaulang; Hsu, Chia-Tse; Ting, Chun-Jen
PATENT ASSIGNEE(S): Academia Sinica, Taiwan
SOURCE: Eur. Pat. Appl., 15 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1090994	A2	20010411	EP 2000-304253	20000519
EP 1090994	A3	20010718		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: US 1999-412558 A 19991005

AB The invention provides a method for making **protein**-based vaccines using a **receptor binding domain** of a **Pseudomonas exotoxin A** or a

functional variant thereof, and at least two copies of a **peptide** sequence. The invention is based on the discovery of a new means of generating an immune response to a **peptide** antigen by concatenating the **peptide** and fusing the concatemer to a **receptor binding domain** of a Pseudomonas exotoxin. Such a fusion **protein** elicits antigen-specific antibodies in a variety of mammals, with little or no toxicity obsd. In particular, the invention provides two new multimeric vaccines, against **vaccinia** virus and against **gonadotropin releasing hormone**, resp.

IT **9034-40-6, Gonadotropin Releasing Hormone**
RL: BSU (Biological study, unclassified); BIOL (Biological study) (vaccines to; method for making fusion **protein** vaccines using **repeat** immunogens and **receptor binding domain** of Pseudomonas exotoxin)

L13 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:152357 CAPLUS
DOCUMENT NUMBER: 134:192236
TITLE: Pseudomonas fusion **protein** vaccines
INVENTOR(S): Hwang, Jaulang; Shang, Huey-fang; Chen, Tzong-yueh
PATENT ASSIGNEE(S): Academia Sinica, Taiwan
SOURCE: Eur. Pat. Appl., 14 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1078988	A1	20010228	EP 1999-306862	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001078765	A2	20010327	JP 1999-243264	19990830

PRIORITY APPLN. INFO.: EP 1999-306862 A 19990827

AB A fusion **protein** suitable as a vaccine is provided that contains at least three Pseudomonas antigens or antigenic fragments. These polypepitde moieties comprise: (1) a **receptor binding domain** of **Pseudomonas exotoxin A** functional variant thereof; (2) a membrane translocation domain of **Pseudomonas exotoxin A** or functional variant thereof; (3) a **Pseudomonas** lipoprotein I or functional variant thereof, or antigenic fragment of a **Pseudomonas** lipoprotein I or functional variant thereof; and (4) an antigenic C-terminal fragment of a **Pseudomonas** porin **protein** F or functional variant thereof. Such a fusion **protein** was constructed comprising (His)6-PE1-405-OprI19-83-OprF24-350 (I): i.e., a histidine affinity tag attached to residues 1-405 of the **Pseudomonas** aeruginosa **exotoxin A**, which is then attached to residues 19-83 of the **Pseudomonas** lipoprotein I, and finally residues 24-350 of **Pseudomonas** porin **protein** F. I induces higher levels of anti-PE antibodies than an immunogen including PE alone, and the antibodies are capable of neutralizing the cytotoxicity of PE on NIH3T3 cells.

I also affords significantly higher protection (80%) against challenge with PE-hyper-producing strain PA103 than OprF alone (40%).

REFERENCE COUNT: 2
REFERENCE(S): (1) Behringwerke Ag; EP 0717106 A 1996 CAPLUS
(2) The Government Of The United States; WO 9902713 A 1999 CAPLUS

L13 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:525737 CAPLUS
DOCUMENT NUMBER: 133:236494
TITLE: Vaccination against **gonadotropin-releasing hormone** (**GnRH**) using toxin **receptor-binding domain**-conjugated **GnRH repeats**
AUTHOR(S): Hsu, Chia-Tse; Ting, Chun-Yuan; Ting, Chun-Jen; Chen, Tzong-Yueh; Lin, Chia-Po; Whang-Peng, Jacqueline; Hwang, Jaulang
CORPORATE SOURCE: Graduate Institute of Life Science, National Defense Medical Center, Institute of Molecular Biology, Academia Sinica, Taipei, 11529, Taiwan
SOURCE: Cancer Res. (2000), 60(14), 3701-3705
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A method for the prepn. of an immunogen contg. multiple copies of a self-**peptide** in linear alignment was designed to overcome the difficulty of inducing an immune response to poorly immunogenic **peptide** antigens. DNA fragments encoding multiple **repeats** of the self-**peptide** were generated by a new technique, termed template-**repeated** polymerase chain reaction (TR-PCR), which could be subcloned into an expression vector for prodn. of **peptide repeats** as an immunogen. This approach was tested by constructing fusion **proteins** contg. the **receptor-binding domain** of **Pseudomonas exotoxin A** and multiple copies of the 10-residue sequence of the **peptide** hormone **gonadotropin-releasing hormone** (**GnRH**). Immunization of female rabbits with the immunogen that contained the exotoxin **receptor-binding domain** and 12 copies of **GnRH** (PEIa-GnRH12) resulted in the generation of high-titer antibodies specific for **GnRH**. Although at equal molar basis of the **GnRH** moiety, the immunogen that contained single copy of **GnRH** (PEIa-GnRH1) induced low-titer anti-**GnRH** antibodies. These observations suggest that the presence of multiple **peptide repeats** is a key factor in eliciting an immune response. In addn., anti-**GnRH** antibodies effectively neutralized **GnRH** activity in vivo, as demonstrated by the degeneration of the ovaries in the injected rabbits. Because anti-**GnRH** antibody could be functionally analogous to **GnRH** antagonist, which has been used to treat patients with ovarian cancer, vaccination of PEIa-GnRH12 presents a potential therapeutic application for the treatment of **GnRH**-sensitive ovarian cancer.

IT **9034-40-6**, LH-RH

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(vaccination against **GnRH** multimer-toxin fusion construct induces neutralizing antibodies to)

REFERENCE COUNT: 16

REFERENCE(S):
(1) Baselga, J; Cancer Res 1998, V58, P2825 CAPLUS
(2) Baselga, J; J Clin Oncol 1996, V14, P737 CAPLUS
(3) Baselga, J; J Natl Cancer Inst 1993, V85, P1327 CAPLUS
(4) Conn, P; Fed Proc 1984, V43, P2351 CAPLUS
(5) Eidne, K; Science (Washington DC) 1985, V229, P989 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:31309 CAPLUS
DOCUMENT NUMBER: 132:74559
TITLE: Crossless retroviral vectors for gene therapy created by reducing its overlapped sequence to gag/pol and env expression vectors
INVENTOR(S): Respess, James G.; Depolo, Nicholas J.; Chada, Sunil; Sauter, Sybille; Bodner, Mordechai; Driver, David A.
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: U.S., 63 pp., Cont.-in-part of U.S. Ser. No. 721,327, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6013517	A	20000111	US 1997-850961	19970505
PRIORITY APPLN. INFO.:			US 1994-240030	19940509
			US 1995-437465	19950509
			US 1996-643411	19960506
			US 1996-721327	19960926

AB Retroviral vector constructs are described which have a 5' LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences, an origin of second strand synthesis and a 3' LTR, wherein the vector construct lacks retroviral gag/pol or env coding sequences. In addn., gag/pol, and env expression-cassettes are described wherein the expression cassettes lack a consecutive sequence of more than 8 nucleotides in common. The above-described retroviral vector constructs, gag/pol and env expression cassettes may be utilized to construct producer cell lines which preclude the formation of replication competent virus. Moloney murine leukemia virus vectors were prepd. and HT1080 and D17 cell lines infected with these vectors were produced. The resulting cell lines produced reduced titers of retroviral vectors depending on degree of sequence overlap among the various vectors. With 2 or 3 areas of sequence overlap eliminated, titers were decreased 5-10-fold; with all overlap eliminated, titers were decreased 5-50-fold.

REFERENCE COUNT: 114

REFERENCE(S): (1) Acsadi; Nature 1991, V352, P815 CAPLUS
(2) Altmann; Nature 1989, V338, P512 CAPLUS
(3) Anon; EP 133123 A1 1985 CAPLUS
(4) Anon; EP 173254 A1 1986 CAPLUS
(5) Anon; JP 01-128788 1989 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:532845 CAPLUS
DOCUMENT NUMBER: 131:267881
TITLE: The **Pseudomonas** aeruginosa **exotoxin A** regulatory gene, ptxS: evidence for negative autoregulation
AUTHOR(S): Swanson, Britta L.; Colmer, Jane A.; Hamood, Abdul N.
CORPORATE SOURCE: Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, TX, 79430, USA
SOURCE: J. Bacteriol. (1999), 181(16), 4890-4895
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have previously described a **Pseudomonas** aeruginosa gene, ptxR, which enhances **exotoxin A** prodn. at the transcriptional level. We have also described another gene, ptxS, which is transcribed divergently from ptxR and interferes with the enhancement of exotoxin A synthesis by ptxR. However, the mechanisms through which ptxR and/or ptxS are regulated is not known. In this study, we attempted (by using the DNA gel shift assay) to det. if P. aeruginosa contains a potential regulatory **protein** that binds specifically to the ptxR or ptxS upstream region. In the initial anal., different-sized gel shift bands were detected when a probe contg. the ptxR-ptxS intergenic region was incubated with the lysate of P. aeruginosa PAO1. The strongest binding activity was detected with a smaller fragment that represents the ptxS upstream region. Addnl. deletion anal. localized the binding to a 52-bp fragment immediately upstream of ptxS. The gel shift band was not detected when the 52-bp fragment was incubated with the lysate of the ptxS isogenic mutant PAO1::ptxS. However, the binding band was regenerated when a plasmid carrying ptxS intact was introduced into PAO1::ptxS. In addn., the gel shift band was detected when the 52-bp fragment was incubated with a lysate of Escherichia coli in which ptxS was overexpressed from the T7 promoter. The effect of PtxS on ptxS expression was examd. by using a ptxS-lacZ fusion plasmid. The level of .beta.-galactosidase activity produced by PAO1::ptxS carrying the fusion plasmid was four- to fivefold higher than that produced by PAO1 carrying the same plasmid. Using DNase I footprinting anal., the binding region was specified to a 20-bp fragment. Within the fragment, a 14-bp palindromic sequence exists that may function as a PtxS binding site. These results suggest that PtxS autoregulates its synthesis by binding to a specific sequence within the ptxS upstream region.
REFERENCE COUNT: 35
REFERENCE(S): (4) Bouchez, D; Plasmid 1991, V25, P27 CAPLUS
(5) Choy, H; Proc Natl Acad Sci USA 1993, V90, P472 CAPLUS

(6) Colmer, J; Mol Gen Genet 1998, V258, P250
CAPLUS
(7) Gambello, M; Infect Immun 1993, V61, P1180
CAPLUS
(8) Gerlach, P; Mol Microbiol 1990, V4, P479
CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:236737 CAPLUS
DOCUMENT NUMBER: 130:232466
TITLE: Molecularly guided medicine comprised of fusion **protein** of interleukin-2(60)-PE40 and its recombinant preparation
INVENTOR(S): Lu, Shengdong; Zhang, Meng; Li, Huanlou
PATENT ASSIGNEE(S): Medical Biological Technology Inst. Chinese Academy of Medical Sciences, Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 45 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1119677	A	19960403	CN 1994-116597	19940929

AB Disclosed is a cell-specific medicine comprised of a fusion **protein** contg. IL-2(60), the N-terminal 60 amino acids encompassing the IL-2 **receptor-binding domain**, and PE40, a mutant form of **Pseudomonas exotoxin A** devoid of its native cell recognition and binding domain and is toxic to IL-2 receptor bearing cells. A recombinant expression vector plasmid pZM10 contg. the fusion **protein**-encoding sequence, an Escherichia coli contg. the vector, and a fusion **protein** expressed by the E.coli are also disclosed.

L13 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:130162 CAPLUS
DOCUMENT NUMBER: 130:332413
TITLE: BR96 sFv-PE40 immunotoxin: nonclinical safety assessment
AUTHOR(S): Haggerty, H. G.; Warner, W. A.; Comereski, C. R.; Peden, W. M.; Mezza, L. E.; Damle, B. D.; Siegall, C. B.; Davidson, T. J.
CORPORATE SOURCE: Department of Drug Safety Evaluation, Bristol-Myers Squibb, Syracuse, NY, 13221, USA
SOURCE: Toxicol. Pathol. (1999), 27(1), 87-94
CODEN: TOPADD; ISSN: 0192-6233
PUBLISHER: Society of Toxicologic Pathologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB BR96 sFv-PE40, a recombinant DNA-derived fusion **protein** composed of the heavy- and light-chain variable region domains of the monoclonal antibody BR96 and the translocation and catalytic domains of **Pseudomonas exotoxin A**, is

being developed for the treatment of solid tumors expressing cell surface Lewisy-related antigens. Single- and **repeat**-dose i.v. toxicity studies in rats and dogs and a comparative ex vivo tissue-binding study with rat, dog, and human tissues were conducted to assess the toxicity of BR96 sFv-PE40 and to est. a safe starting dose in humans. Addnl. studies were performed to investigate the prevention of pulmonary vascular-leak syndrome, the dose-limiting toxicity of BR96 sFv-PE40 in rats, and the immunogenicity of BR96 sFv-PE40. In single-dose studies in rats, the vascular leak appeared to be primarily confined to the lungs; however, with a **repeat**-dose regimen (every other day for 5 doses) other organs including the brain and heart were involved at LDs (12-15 mg/m2 cumulative). Single doses of 1.8 mg/m2 and a cumulative 3.8 mg/m2 dose (0.75 mg/m2, every other day for 5 doses) were generally well tolerated in rats. These doses are greater than doses required to cure rodents bearing human tumor xenografts. In dogs, the major target organ following single or **repeated** doses (every 3 days for 5 doses) was the pancreas. Morphol. changes in the exocrine pancreas ranged from atrophy with single-cell necrosis to diffuse acinar necrosis. After a 1-mo dose-free observation period, no residual pancreatic toxicity was obsd. in dogs given single doses up to 6.0 mg/m2 or 5 doses of 2.4 mg/m2 (12 mg/m2 cumulative). No pancreatic toxicity was obsd. at doses <0.6 mg/m2 in high Lewisy-expressing dogs. Assessment of trypsin-like immunoreactivity was useful in monitoring changes in pancreatic function. The immunogenicity of BR96 sFv-PE40 could be inhibited by combined treatment with an immunosuppressant in dogs, thus maintaining exposure to BR96 sFv-PE40.

REFERENCE COUNT: 17

REFERENCE(S):
(2) Friedman, P; Cancer Res 1993, V53, P334 CAPLUS
(3) Ghetie, V; Pharmacol Ther 1994, V63, P209 CAPLUS
(10) Siegall, C; Clin Cancer Res 1997, V3, P339 CAPLUS
(11) Siegall, C; J Immunol 1994, V152, P2377 CAPLUS
(13) Siegall, C; Proc Natl Acad Sci USA 1994, V91, P9514 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:23921 CAPLUS
DOCUMENT NUMBER: 130:192473
TITLE: Renaturation and detection cytobiological activity of recombinant receptor-binding **protein** of **exotoxin A** of **Pseudomonas** aeruginosa
AUTHOR(S): Liu, Xiaoming; Ma, Conglin; Guo, Xuejun; Zhu, Ping; Wang, Jinqi; Zhen, Yingkai
CORPORATE SOURCE: Mil. Vet. Inst., Univ. Agric. Anim. Sci., Changchun, 130062, Peop. Rep. China
SOURCE: Zhongguo Shouyi Xuebao (1998), 18(5), 469-472
CODEN: ZSXUF5; ISSN: 1005-4545
PUBLISHER: Zhongguo Shouyi Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The purified recombinant receptor-binding **protein** of

exotoxin A of P. aeruginosa expressed in E. coli was renatured preliminarily by dild. progressively dialysis the renatured sol. **protein** was used in the expt. of competitive inhibition for cytotoxicity of PEA. 10 .mu.G/L PEA incubated with its sensitive cell line L929, about 36 h later, the cytobiol. pathol. change could be found under microscope, but the cell which incubated PEA and renatured recombinant receptor-binding **protein** of PEA was the same as the cell incubated without PEA, which was still viability, division and proliferation until all the cell was aging and death. It is indicated that the action of cytotoxicity of PEA may be inhibited by the recombinant receptor-binding **protein** of PEA.

L13 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:228309 CAPLUS
DOCUMENT NUMBER: 128:279348
TITLE: Purification of recombinant **protein** containing **receptor-binding domain** of **exotoxin A** of **Pseudomonas** aeruginosa
AUTHOR(S): Liu, Xiaoming; Guo, Xuejun; Ma, Conglin; Zhu, Ping; Meng, Ruiqi; Li, Jiping
CORPORATE SOURCE: Military Veterinary Inst., Univ. Agriculture and Animal Sci., Changchun, 130062, Peop. Rep. China
SOURCE: Zhongguo Shouyi Xuebao (1998), 18(1), 38-41
CODEN: ZSXUF5; ISSN: 1005-4545
PUBLISHER: Zhongguo Shouyi Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Expressing plasmid contg. **receptor-binding domain** of exotoxin A of P. aeruginosa (PEA), pET-EAB, was transformed into E. coli BL21(DE3). By inducing of IPTG, a recombinant **protein** contg. **receptor-binding domain** of PEA, named PE34, was expressed. PE34 formed inclusion body in expressed E. coli. The expressed E. coli was lyzed by lysozyme-deoxycholic acid sodium and supersonic. The inclusion body was prepd. by centrifugation and washing with 2 mol/L urea with the purifn. rate of the inclusion body being above 75%. Then it was dissolved by 8 mol/L urea and further purified by Sephacryl S-200 gel filter and DEAE-Sepharose Fast Flow ion-exchange chromatog. The purified PE34 appeared a single band in SDS-PAGE gel with its purifn. rate and recovery rate being 95.8% and 24.5%.

L13 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:130296 CAPLUS
DOCUMENT NUMBER: 128:253489
TITLE: Cloning of **Pseudomonas exotoxin A** receptor binding subunit gene and its expression in Escherichia coli
AUTHOR(S): Guo, Xuejun; Liu, Xiaoming; Zhu, Ping; Liu, Zi; Meng, Ruiqi; Ma, Conglin; Feng, Shuzhang
CORPORATE SOURCE: Mil Vet Inst., Univ. Agric. Animal Sci., Changchun, 130062, Peop. Rep. China
SOURCE: Zhongguo Shouyi Xuebao (1997), 17(3), 226-229
CODEN: ZSXUF5; ISSN: 1005-4545
PUBLISHER: Zhongguo Shouyi Xuebao Bianjibu
DOCUMENT TYPE: Journal

LANGUAGE: Chinese
AB For the purpose of treatment of **Pseudomonas exotoxin A**-induced diseases, the **receptor -binding domain** and partial membrane domain of the structure gene of **Pseudomonas exotoxin A** (PEA) (a 1,000 bp DNA segment encoding 309 amino acids) was cloned and expressed under the control of the T7 promotor. Analyzed by SDS-PAGE the mol. wt. of the **protein** expressed was about 34,000, similar to the putative Mr. and was called PE34. Western-blotting showed that FE34 could react with anti-PEA serum indicating that PE34 should be the target **protein**. SDS-PAGE TLC-scanning showed that PE34 accounted for 56% of the total bacteria **protein**.

L13 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:260787 CAPLUS
DOCUMENT NUMBER: 120:260787
TITLE: Targeted therapy with immunotoxins in a nude rat model for leptomeningeal growth of human small cell lung cancer
AUTHOR(S): Myklebust, Arne Thormod; Godal, Aslak; Fodstad, Oeystein
CORPORATE SOURCE: Inst. Cancer Res., Norweg. Radium Hosp., Oslo, Norway
SOURCE: Cancer Res. (1994), 54(8), 2146-50
CODEN: CNREA8; ISSN: 0008-5472
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Metastasis to the central nervous system in patients with small cell lung cancer is not uncommon, and a fraction of the cases have leptomeningeal disease for which no effective therapy is available. To establish an exptl. model for evaluation of new therapeutic approaches for such tumor lesions, 1 .times. 106 human H-146 cells were injected directly into the cerebrospinal fluid in the cisterna magna of nude rats. Small, superficial leptomeningeal tumors developed, consistently resulting in symptoms of central nervous system involvement after a mean latency of 20 days. The model was used to study the efficacy of intrathecal targeted therapy with immunotoxins. The monoclonal anti-carcinoma antibodies MOC-31 and NrLu10 and the growth factor transferrin were conjugated to **Pseudomonas exotoxin A** (PE), and 1 day after tumor cell inoculation instilled in the cisterna magna as a single bolus dose of 1.5 .mu.g. The antibody conjugates, which were highly cytotoxic to target cells in a **protein** synthesis inhibition assay in vitro, increased the symptom-free latency by 35-46%. PE had no effect, reflecting a lower in vitro cytotoxicity and possibly also a down-regulation of transferrin-receptor expression in the meningeal H-146 tumors. Delayed or **repeated** treatment with MOC-31-PE was less effective than day 1 administration, whereas the addn. of 10% glycerol to the injection soln. increased the symptom-free period to 72%. The efficacy of MOC-31-PE is superior to reported effects obtained in similar models with other therapies, and the results support the development of this immunotoxin towards clin. evaluation in small cell lung cancer patients with leptomeningeal carcinomatosis.

L13 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:29457 CAPLUS

DOCUMENT NUMBER: 120:29457
TITLE: Development of derivatives of **exotoxin A** of **Pseudomonas** aeruginosa which contain either the fragment of **protein** A of Staphylococcus or interleukin 2 and study on stability of these **proteins** in Escherichia coli cells
AUTHOR(S): Zdanovsky, A. G.; Zdanovska, M. V.; Yankovsky, N. K.; Debabov, V. G.
CORPORATE SOURCE: Inst. for Genet. Select. Ind. Microorg., Moscow, 113545, Russia
SOURCE: Biotekhnologiya (1993), (6), 15-20
CODEN: BTKNEZ; ISSN: 0234-2758
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB Derivs. of exotoxin A lacking the **receptor-binding domain** are nontoxic for eukaryotic cells. However, such derivs. can be used as the catalytic components of immunotoxins. To create such immunotoxins, exotoxin A derivs. should be fused to **proteins** which recognize specific receptors of eukaryotic cells. Hybrid **proteins** were constructed by gene fusion between fragments of exotoxin A and fragments of staphylococcal **protein** A or interleukin 2. Anal. of these **proteins** showed that, with one exception, all are stable in E. coli.

L13 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:617398 CAPLUS
DOCUMENT NUMBER: 119:217398
TITLE: Desensitization to specific allergens with interleukin-4 receptor-binding fusion **protein**
INVENTOR(S): Waters, Cory Ann; Nichols, Jean C.
PATENT ASSIGNEE(S): Seragen, Inc., USA
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9315766	A1	19930819	WO 1993-US1034	19930204
W: AU, CA, FI, JP, KR, NO, NZ				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9336580	A1	19930903	AU 1993-36580	19930204
PRIORITY APPLN. INFO.:			US 1992-832843	19920210
			WO 1993-US1034	19930204

AB A method is disclosed for desensitizing an animal to a particular antigen, wherein at or about a time of exposure of the animal to the allergen, a mol. is administered which specifically binds to interleukin-4 (IL-4) receptor expressed on a peripheral blood mononuclear cell (PBMC) of the animal, and is capable of decreasing the viability of the PBMC to which it binds. Thus, DAB389IL-4 (a fusion **protein** in which the **receptor-binding domain** of diphtheria toxin has been replaced by human IL-4) was prepd. with std. recombinant DNA

methodol. DAB389IL-4 eliminated IgE secretion by B cells undergoing Ig class switching, but did not eliminate IgE secretion by B-cells (from an atopic patient) which had already undergone an Ig class switch.

L13 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:185521 CAPLUS
DOCUMENT NUMBER: 118:185521
TITLE: Residues 1-254 of anthrax toxin lethal factor are sufficient to cause cellular uptake of fused **polypeptides**
AUTHOR(S): Arora, Naveen; Leppla, Stephen H.
CORPORATE SOURCE: Lab. Microb. Ecol., Natl. Inst. Dent. Res., Bethesda, MD, 20892, USA
SOURCE: J. Biol. Chem. (1993), 268(5), 3334-41
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anthrax lethal toxin is a complex of protective antigen (PA, 735 amino acids) and lethal factor (LF, 776 amino acids) that lyses certain eukaryotic cells. LF interacts with PA to gain access to the cytosol to assert its toxicity. The internalization of LF requires that PA bind to a specific membrane receptor and be cleaved by a cell-surface protease (probably furin), so as to expose a site on PA to which LF binds with high affinity. To localize LF functional domains, amino, carboxyl, and internal deletions of LF were made. Toxicity was eliminated by deletion of 40 and 47 residues from the amino and carboxyl termini, resp. Similarly, deleting the first of the four imperfect **repeats** of 19 amino acids located at residues 308-383 made LF non-toxic, showing that this region is also essential for activity. To identify the min. region of LF which is required for binding to PA, varying amino-terminal portions of LF were fused to the ADP-ribosylation domain of **Pseudomonas exotoxin A**. Fusion **proteins** contg. residues 1-254 of LF were toxic when administered with PA, while those having only residues 1-198 of LF were inactive, showing that the PA-binding domain of LF lies within residues 1-254.

L13 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:99766 CAPLUS
DOCUMENT NUMBER: 118:99766
TITLE: **Protein** engineering of DAB-IL-2 fusion toxins to increase biological potency
AUTHOR(S): Kiyokawa, Tetsuyuki; Williams, Diane P.; Snider, Catherine E.; Waters, Cory A.; Nichols, Jean C.; Strom, Terry B.; Murphy, John R.
CORPORATE SOURCE: Med. Cent., Boston Univ., Boston,
SOURCE: Ann. N. Y. Acad. Sci. (1991), 636(Clone-Specific Immunoregul.), 331-
CODEN: ANYAA9; ISSN: 0077-8923
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 43 refs. The genetic replacement of ei native diphtheria toxin or **Pseudomonas exotoxin A receptor binding domain** with the eukaryotic cell receptor-specific **polypeptide** horm or growth factor sequences has resulted in the develop

class of biol. response modifier-the fusion toxin. The 1st of these fusion toxins, DAB486-interleukin-2 (IL-2), is currently in human phase I clin. trials and the early results clearly demonstrate that this mol. is safe, well-tolerated, and biol. active in the elimination of high-affinity IL-2 receptor-pos. leukemia and lymphoma cells without adverse side effect. DAB486-IL-2 is a bipartite fusion **protein** composed of diphtheria toxin fragment A and fragment B sequences to Ala486 linked to Pro2 through Thr133 of human IL-2. This chimeric **protein** is the product of a genetic fusion between a truncated gene encoding fragment A and the membrane-assocg. domains of fragment B of diphtheria toxin and a synthetic gene encoding human IL-2. DAB486-IL-2 has been shown to selectively bind to high-affinity IL-2 receptors, be internalized by receptor-mediated endocytosis, and facilitate the delivery of diphtheria toxin fragment A to the cytosol of target cells. Recent studies have defined the minimal size of fragment B that is required to deliver fragment A across the endocytic vesicle membrane in target cells, and defined the site of proteolytic processing involved in the release of fragment A from the intact fusion toxin mol.

L13 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:242486 CAPLUS
DOCUMENT NUMBER: 114:242486
TITLE: Functional analysis of **exotoxin A**-related **protein** of **Pseudomonas** aeruginosa lacking residues 225-412
AUTHOR(S): Guidi-Rontani, Chantal
CORPORATE SOURCE: Dep. Microbiol. Mol. Genet., Harvard Med. Sch., Boston, MA, USA
SOURCE: FEMS Microbiol. Lett. (1991), 80(1), 103-9
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The crystal structure of the exotoxin A (ETA) of P. aeruginosa showed that this **protein** is folded into three distinct domains. Domain I (Ia and Ib), the amino-terminal domain, is the **receptor-binding domain** of ETA and domain III, the carboxy-terminal domain, is responsible for the ADP-ribosyl transferase activity of the toxin. To elucidate the function(s) of domains 1b and II in the intoxication process and to define the region of the domain III necessary for ADP-ribosylating activity, a defined deletion in the structural gene of P. aeruginosa ETA encompassing residues 225-412 was constructed and an ETA-related product, DeID (from which all of domains II and Ib were deleted), was expressed. The ETA-related **protein** did not penetrate sensitive cells, but retained the same specific activity to ADP-ribosylate elongation factor-2 as wild-type toxin. This suggests that domain II is necessary to allow toxin internalization by sensitive cells and that the absence of domain Ib does not interfere with enzymic activity. The domain strictly involved in ADP-ribosylation activity encompasses residues 412-613.

L13 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1990:93571 CAPLUS
DOCUMENT NUMBER: 112:93571
TITLE: Pseudomonas exotoxin contains a specific

sequence at the carboxyl terminus that is required for cytotoxicity

AUTHOR(S): Chaudhary, Vijay K.; Jinno, Yoshihiro; FitzGerald, David; Pastan, Ira

CORPORATE SOURCE: Lab. Mol. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1990), 87(1), 308-12
CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Pseudomonas exotoxin (PE), a single-chain **polypeptide** toxin of 613 amino acids, consists of 3 functional domains: an amino-terminal **receptor-binding domain**, a middle translocation domain, and a carboxyl-terminal ADP-ribosylation domain. Deletion of as few as 2 or as many as 11 amino acids from the carboxyl terminus of PE does not affect ADP-ribosylation activity but produces noncytotoxic mols. Deletions and substitutions between positions 602 and 611 of PE show that the last 5 amino acids of PE are very important for its cytotoxic action. The carboxyl-terminal sequence of PE is Arg-Glu-Asp-Leu-Lys. Mutational anal. indicates that a basic amino acid at 609, acidic amino acids at 610 and 611, and a leucine at 612 are required for full cytotoxic activity. Lysine at 613 can be deleted or replaced with arginine but not with several other amino acids. Mutant toxins are able to bind normally to target Swiss mouse 3T3 cells and are internalized by endocytosis, but apparently they do not penetrate into the cytosol. A PE mol. that ends with Lys-Asp-Glu-Leu, which is a well defined endoplasmic reticulum retention sequence, is fully cytotoxic, suggesting that a common factor may be involved in intoxication of cells by PE and retention of **proteins** in the lumen of the endoplasmic reticulum. Sequences similar to those at the carboxyl end of PE are also found at the end of cholera toxin A chain and Escherichia coli heat-labile toxin A chain.

L13 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1985:469507 CAPLUS

DOCUMENT NUMBER: 103:69507

TITLE: Enzyme-linked immunosorbent assay for detection of antibodies to Pseudomonas aeruginosa exoproteins

AUTHOR(S): Granstroem, M.; Wretlind, B.; Markman, B.; Pavlovskis, O. R.; Vasil, M. L.

CORPORATE SOURCE: Dep. Bacteriol., Natl. Bacteriol. Lab., Stockholm, S-10521, Swed.

SOURCE: Eur. J. Clin. Microbiol. (1985), 4(2), 197-200
CODEN: EJCMDM; ISSN: 0722-2211

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enzyme-linked immunosorbent assays were developed with 4 purified P. aeruginosa extracellular **proteins** (exotoxin A, elastase, alk. protease, and phospholipase C) to det. antibody levels in sera from healthy subjects and the serol. response in patients colonized or infected with P. aeruginosa. Five of 39 burn patients with wounds colonized by P. aeruginosa had elevated antibody titers to alk. protease. Response to the other antigens was found in only a few patients. P. aeruginosa Infections (septicemia, ostetis,

pneumonia etc.) resulted in increased antibody levels to exotoxin A or phospholipase C in 15 of 22 patients. These findings suggest that **repeated** detns. of antibodies to P. aeruginosa **exotoxin A** and phospholipase C might be used to monitor therapy in certain patients with osteitis and other **Pseudomonas** infections.

(FILE 'MEDLINE,' BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 14:57:12 ON 13 NOV 2001)

L14 23 S L11
L15 30 S L12
L16 49 S L14 OR L15
L17 28 DUP REM L16 (21 DUPLICATES REMOVED)

L17 ANSWER 1 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-257973 [26] WPIDS
DOC. NO. CPI: C2001-077773
TITLE: Targeting compounds typically lethal factor **polypeptide** to cells for prophylactic by using mutant protective antigen **proteins** that target cells containing high amounts of cell-surface metalloproteinases or plasminogen activators.
DERWENT CLASS: B04 D16
INVENTOR(S): BUGGE, T; HANSEN-BIRKEDAL, H; LEPPLA, S H; LIU, S; NETZEL-ARNETT, S
PATENT ASSIGNEE(S): (USSH) US DEPT HEALTH & HUMAN SERVICES
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001021656 A2		20010329	(200126)*	EN	77
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2001025725 A		20010424	(200141)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001021656 A2		WO 2000-US26192	20000922
AU 2001025725 A		AU 2001-25725	20000922

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001025725 A	Based on	WO 200121656

PRIORITY APPLN. INFO: US 1999-155961 19990924
AN 2001-257973 [26] WPIDS
AB WO 200121656 A UPAB: 20010515

NOVELTY - Targeting (M1) a compound (C) to cells over-expressing matrix metalloproteinases (MMP), plasminogen activators (PT) or a PT receptor is new.

DETAILED DESCRIPTION - (M1) comprises:

(1) administering to the cell a mutant protective antigen **protein** (PA) comprising a MMP or a PT-recognized cleavage site in place of the native protective antigen furin-recognized cleavage site, where the mutant protective antigen is cleaved by a MMP or a plasminogen activator; and

(2) administering to the cell a compound comprising a lethal factor **polypeptide** comprising a protective antigen binding site; where the lethal factor **polypeptide** binds to cleaved protective antigen and is translocated into the cell, thereby delivering the compound to the cell

An INDEPENDENT CLAIM is also included for an isolated mutant PA in which native PA furin-recognized cleavage site is replaced by sequences specifically cleaved by MMPs or PTs.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Inducer of cytotoxic events. To test that PA mutants (PA-L1 and PA-L2) only kill MMP expressing tumor cells but not MMP non-expressing normal cells, 3 human tumor cell lines, fibrosarcoma HT1080, melanoma A2058 and breast cancer MDA-MB-231 and one non-tumor cell line Vero, were employed in cytotoxicity assay. Cytotoxicity of wild type PA (WT-PA) and PA mutants to these cells were performed onto 96-well plates. Different concentrations of WT-PA, PA-L1 and PA-L2 combined with FP59 were separately added to the cells and challenged the cells for 6 and 48 hours. Cytotoxicity was allowed to develop for 48 hours. The EC50 of PA and PA mutants was determined. The results showed that MMP non-expressing Vero cells were quite resistant to PA-L1 and PA-L2 but very sensitive to wild-type PA with dose-dependent manner. PA-L1 and PA-L2 nicked by MMP-2 in vitro efficiently killed Vero cells even with 6 hours toxin challenge in dose-dependent manner, demonstrating the non toxicity of PA-L1 and PA-L2 to Vero cells was due to Vero cells lacking the ability of processing them into the active form PA63. The two MMP expressing tumor cells, HT1080, A2058 and MDA-MB-231, were susceptible to WT-PA as well as PA-L1 and PA-L2 and the sensitivity of these PA mutants directly correlated with the overall expression levels of MMPs of the tumor cells.

USE - The method is useful for targeting compounds, especially a native lethal factor (LF) or LF fusion **protein**, fused to another compound to a cell over-expressing MMP, PT or PT receptor. The fusion is typically chemical or recombinant. Compounds fused to LF include a diagnostic or therapeutic agent, shiga toxin, A chain of diphtheria toxin or **Pseudomonas exotoxin A**, a detectable moiety or a nucleic acid. The cell is especially an inflammatory or cancer cell, including lung, breast, bladder, thyroid, liver, pleural, pancreatic, ovarian, cervical, colon cancer, fibrosarcoma, neuroblastoma, glioma, melanoma, monocytic leukemia or myelogenous leukemia (claimed). PA containing **proteins** and lethal factor containing **proteins** are administered directly to a patient e.g. for inhibition of cancer, tumor or precancer cells in vivo.

ADVANTAGE - The method facilitates killing of tumor cells without serious damage to normal cells.

Dwg.0/17

L17 ANSWER 2 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2001-309780 [33] WPIDS
DOC. NO. CPI: C2001-095841
TITLE: New **polypeptides** having multiple copies of a **peptide** antigen fused to the **receptor binding domain** of a Pseudomonas exotoxin, useful as a vaccine and for generating antibodies for diagnostic and/or therapeutic procedures.
DERWENT CLASS: B04 D16
INVENTOR(S): HSU, C; HWANG, J; TING, C
PATENT ASSIGNEE(S): (SINI-N) ACAD SINICA; (SINI-N) ACAD SINICA INC
COUNTRY COUNT: 28
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1090994	A2	20010411	(200133)*	EN	15
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
AU 2000062500	A	20010412	(200133)		
CA 2304377	A1	20010405	(200133)	EN	
NZ 507368	A	20010629	(200140)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1090994	A2	EP 2000-304253	20000519
AU 2000062500	A	AU 2000-62500	20001005
CA 2304377	A1	CA 2000-2304377	20000428
NZ 507368	A	NZ 2000-507368	20001005

PRIORITY APPLN. INFO: US 1999-412558 19991005
AN 2001-309780 [33] WPIDS
AB EP 1090994 A UPAB: 20010615
NOVELTY - A new **polypeptide** comprises a **receptor binding domain** of a **Pseudomonas exotoxin A** or its functional variant; and at least two copies of a **peptide** sequence.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid (N1) encoding the **polypeptide**;

(2) a method of producing the **polypeptide**; and

(3) a vaccine composition comprising at least one **polypeptide** or at least nucleic acid cited above, and optionally a pharmaceutical carrier.

ACTIVITY - Immunostimulant.

Mice and pig were immunized with PEIa-**GnRH** 12 (a PEIa plasmid expressing 12 **repeats** of **gonadotropin releasing hormone** (**GnRH**)). The mice received a 100 mu l bolus containing 10 mu g PEIa-**GnRH** 12 and 12 mu g aluminum phosphate for each injection. In addition, a 24 day-old pig was injected once with a 1 ml bolus containing 10 mg PEIa-**GnRH** 12 and 250 mu g aluminum phosphate. **GnRH** -specific antibodies were readily elicited in the mice and pig, indicating that the antigens can elicit an immune response in a variety of animals.

MECHANISM OF ACTION - Vaccine.
USE - The **polypeptide** is useful as a vaccine. The **polypeptide** is useful for generating antibodies that specifically bind a monomeric **peptide** sequence. Such antibodies are useful in diagnostic and/or therapeutic procedures that require the enhancement, inhibition or detection of any molecule that contains the epitope presented by the **peptide** sequence.
Dwg.0/3

L17 ANSWER 3 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-212737 [22] WPIDS
DOC. NO. CPI: C2001-063588
TITLE: New **polypeptide**, useful as vaccines for eliciting antibodies and/or cell-mediated immunity against Pseudomonas bacteria in an animal, comprises a Pseudomonas exotoxin segment and two Pseudomonas outer membrane **protein** segments.
DERWENT CLASS: B04 D16
INVENTOR(S): CHEN, T; HWANG, J; SHANG, H
PATENT ASSIGNEE(S): (SINI-N) ACAD SINICA
COUNTRY COUNT: 26
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1078988	A1	20010228	(200122)*	EN	14
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2001078765 A		20010327	(200133)#		32

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1078988	A1	EP 1999-306862	19990827
JP 2001078765 A		JP 1999-243264	19990830

PRIORITY APPLN. INFO: EP 1999-306862 19990827; JP 1999-243264 19990830
AN 2001-212737 [22] WPIDS
AB EP 1078988 A UPAB: 20010421
NOVELTY - A **polypeptide** (I) comprising:
(a) a **receptor binding domain** (a1) and a membrane translocation domain (a2) of a Pseudomonas exotoxin or the functional variants of (a1) and (a2);
(b) a Pseudomonas lipoprotein I, its antigenic fragment, or the functional variants of the lipoprotein I or fragment; and
(c) an antigenic C-terminal fragment of a Pseudomonas porin **protein** F or its functional variant, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a nucleic acid (II) encoding (I);
(2) a vector (III) containing (II);
(3) a cell (IV) containing (II) or (III);
(4) vaccine compositions comprising at least (I), (II), (III)

or (IV) and optionally a pharmaceutical carrier; and
(5) producing (I).
ACTIVITY - Antibiotic.
MECHANISM OF ACTION - Vaccine.
Three groups of BALB/c mice were immunized with fusion **protein** (PEIF), OprF and **Pseudomonas** aeruginosa **exotoxin A** (PE). The immune responses of the BALB/c mice after three doses of vaccine were determined by enzyme linked immunosorbant assay (ELISA). Results showed that the fusion **protein** induced a vigorous antibody response. PEIF unexpectedly elicited higher levels of anti-PE antibodies than an immunogen including PE alone.
USE - The **polypeptide** is useful in vaccine compositions. The vaccine is useful for eliciting antibodies and/or cell-mediated immunity against Pseudomonas bacteria in an animal (claimed).
Dwg.0/0

L17 ANSWER 4 OF 28 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001423622 MEDLINE
DOCUMENT NUMBER: 21247217 PubMed ID: 11348771
TITLE: A recombinant chimera composed of **repeat** region RR1 of Mycoplasma hyopneumoniae adhesin with Pseudomonas exotoxin: in vivo evaluation of specific IgG response in mice and pigs.
AUTHOR: Chen J R; Liao C W; Mao S J; Weng C N
CORPORATE SOURCE: Department of Pathobiology, Pig Research Institute Taiwan, P.O. Box 23, 35099, ROC, Chunan Miaoli, Taiwan.
SOURCE: VETERINARY MICROBIOLOGY, (2001 Jun 22) 80 (4) 347-57. Journal code: XBW; 7705469. ISSN: 0378-1135.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010730
Last Updated on STN: 20010730
Entered Medline: 20010726

AB Using the binding and translocation domain of **Pseudomonas exotoxin A** [domain III deleted PE termed PE(DeltaIII)] as a vehicle, this study characterized and evaluated a novel application of PE toxin in Mycoplasma hyopneumoniae adhesin used as an immunogen. PCR and sequence analysis revealed that 16 copies of AAKPV(E) in tandem **repeat** region 1 (RR1) of M. hyopneumoniae 97kDa adhesion were successfully fused to the downstream of PE(DeltaIII) to create a subunit vaccine, i.e. PE(DeltaIII)-RR1. This chimeric **protein**, over-expressed in inclusion bodies of E. coli BL21(DE3)pLysS, was characterized by a monoclonal antibody (MAb) F2G5 prepared against RR1 of the 97kDa adhesin and was readily purified. The data indicated that the epitope recognized by MAb F2G5 was located in the structure of PE(DeltaIII)-RR1. Using ELISA and Western blot analyses, the specific IgG immune response against RR1 and whole adhesin in mice immunized with PE(DeltaIII)-RR1 was found more marked than that in mice immunized with the M. hyopneumoniae whole cells. Similarly, PE(DeltaIII)-RR1 also stimulated a remarkable IgG response against RR1 in pigs compared to that in pigs immunized with the conventional

M. hyopneumoniae vaccine. The PE(DeltaIII)-RR1 would be potentially useful for the future development of a M. hyopneumoniae adhesin vaccine.

L17 ANSWER 5 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-159877 [14] WPIDS
CROSS REFERENCE: 1997-558992 [51]
DOC. NO. CPI: C2000-049871
TITLE: New retroviral construct, used to produce retroviral particles for gene therapy, containing a gag/pol sequence that includes at least two stop codons, incapable of producing replicable virus by recombination.
DERWENT CLASS: B04 D16
INVENTOR(S): BODNER, M; CHADA, S; DEPOLO, N J; DRIVER, D A; RESPESS, J G; SAUTER, S
PATENT ASSIGNEE(S): (CHIR) CHIRON CORP
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6013517	A	20000111	(200014)*		63

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 6013517	A	CIP of	US 1994-240030	19940509
		CIP of	US 1995-437465	19950509
		CIP of	US 1996-643411	19960506
		CIP of	US 1996-721327	19960926
			US 1997-850961	19970505

PRIORITY APPLN. INFO: US 1997-850961 19970505; US 1994-240030 19940509; US 1995-437465 19950509; US 1996-643411 19960506; US 1996-721327 19960926
AN 2000-159877 [14] WPIDS
CR 1997-558992 [51]
AB US 6013517 A UPAB: 20000320
NOVELTY - Retroviral vector construct (A) comprises a 5'-LTR (long terminal **repeat**); a tRNA binding site; origin of second strand DNA synthesis; a 3'-LTR and gag/pol sequences (I) modified to contain two or more stop codons.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a producer cell line comprising:

(a) a gag/pol expression cassette; and

(b) an env expression cassette and a retroviral vector construct (A') in which the 3'-end of the gag/pol gene is not homologous with the 5'-end of the env gene, and the 3'-end of the env gene is not homologous with (A'), provided that (A') overlaps with at least 4 nucleotides (nt) at the 5'-end of the gag/pol gene.

ACTIVITY - Anticancer; antiviral; immunomodulatory.

MECHANISM OF ACTION - None given.

USE - (A) are used to produce recombinant retroviral particles for use in gene transfer, particularly gene therapy, e.g. to deliver heterologous sequences that encode cytotoxins, prodrug activators,

replacement genes, antisense sequences or ribozymes, immune accessory molecules and viral immunogens, particularly for treatment or prevention of tumors, viral infections and genetic disorders.

ADVANTAGE - (A) can not generate replication-competent virus by recombination.

Dwg.0/22

L17 ANSWER 6 OF 28 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2000402342 MEDLINE
DOCUMENT NUMBER: 20374289 PubMed ID: 10919636
TITLE: Vaccination against **gonadotropin-releasing hormone (GnRH)** using toxin **receptor-binding domain**-conjugated **GnRH repeats**.
AUTHOR: Hsu C T; Ting C Y; Ting C J; Chen T Y; Lin C P; Whang-Peng J; Hwang J
CORPORATE SOURCE: Graduate Institute of Life Science, National Defense Medical Center, Academia Sinica, Taipei, Taiwan.
SOURCE: CANCER RESEARCH, (2000 Jul 15) 60 (14) 3701-5. Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000824

AB A method for the preparation of an immunogen containing multiple copies of a self-**peptide** in linear alignment was designed in order to overcome the difficulty of inducing an immune response to poorly immunogenic **peptide** antigens. DNA fragments encoding multiple **repeats** of the self-**peptide** were generated by a new technique, termed template-**repeated** polymerase chain reaction (TR-PCR), which could be subcloned into an expression vector for production of **peptide repeats** as an immunogen. This approach was tested by constructing fusion **proteins** containing the **receptor-binding domain** of **Pseudomonas exotoxin A** and multiple copies of the 10-residue sequence of the **peptide** hormone **gonadotropin-releasing hormone** (**GnRH**). Immunization of female rabbits with the immunogen that contained the exotoxin **receptor-binding domain** and 12 copies of **GnRH** (PEIa-GnRH12) resulted in the generation of high-titer antibodies specific for **GnRH**. Although at equal molar basis of the **GnRH** moiety, the immunogen that contained single copy of **GnRH** (PEIa-GnRH1) induced low-titer anti-**GnRH** antibodies. These observations suggest that the presence of multiple **peptide repeats** is a key factor in eliciting an immune response. In addition, anti-**GnRH** antibodies effectively neutralized **GnRH** activity in vivo, as demonstrated by the degeneration of the ovaries in the injected rabbits. Because anti-**GnRH** antibody could be functionally analogous to **GnRH** antagonist, which has been used to treat patients with ovarian cancer, vaccination of PEIa-GnRH12 presents a potential therapeutic

application for the treatment of **GnRH**-sensitive ovarian cancer.

L17 ANSWER 7 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-590695 [50] WPIDS
DOC. NO. NON-CPI: N1999-435671
DOC. NO. CPI: C1999-172440
TITLE: Production of cytotoxic heteromeric **protein** combinatorial libraries, useful for ability to specifically bind to and kill a target cell.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): BRAY, M R; GARIEPY, J
PATENT ASSIGNEE(S): (ONTA-N) ONTARIO CANCER INST
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9940185	A1	19990812	(199950)*	EN	61
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
CA 2222993	A1	19990804	(200004)	EN	
AU 9915530	A	19990823	(200005)		
EP 1051482	A1	20001115	(200059)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9940185	A1	WO 1998-CA1137	19981208
CA 2222993	A1	CA 1998-2222993	19980204
AU 9915530	A	AU 1999-15530	19981208
EP 1051482	A1	EP 1998-959689	19981208
		WO 1998-CA1137	19981208

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9915530	A	Based on	WO 9940185
EP 1051482	A1	Based on	WO 9940185

PRIORITY APPLN. INFO: CA 1998-2222993 19980204
AN 1999-590695 [50] WPIDS
AB WO 9940185 A UPAB: 19991201
NOVELTY - A binding subunit of a wild type heteromeric cytotoxic **protein** is mutated to create a library of microorganism clones producing mutant **proteins** where are then screened for their ability to specifically bind to and kill a target cell.
DETAILED DESCRIPTION - A method for identifying cytotoxic mutant **proteins** capable of binding to a target cell comprises:
(a) selecting a heteromeric **protein** toxin having a

toxic subunit and a binding subunit;

(b) generating a library of microorganism clones producing variant **protein** toxins of the heteromeric **protein** toxin by incorporating mutations into the binding subunit DNA of the heteromeric **protein** toxin; and

(c) screening the variant **protein** toxins of the library against the target cell by isolating clones or pools of clones producing the variant **protein** toxins, treating preparations of the target cells with the variant **protein** toxins and selecting a cytotoxic mutant **protein** or pool of cytotoxic mutant **proteins** that inhibit or kill the target cell.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of killing or inhibiting a target cell comprising treating the target cell with the cytotoxic mutant **protein** or pool of **proteins** from above;

(2) a method for identifying therapeutic **proteins** having binding specificity for a target cell; and

(3) a method for constructing diagnostic probes for detecting the presence of a cell surface marker.

USE - Cytotoxic mutant **proteins** identified by the method can be used to identify therapeutic **proteins** and medicaments having binding specificity for a target cell. The cytotoxic mutants can also be used to construct diagnostic probes for detecting the presence of cell surface markers. These medicaments can be used to target medicines to target cells in host organisms. (All Claimed).

Dwg.0/6

L17 ANSWER 8 OF 28 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 1999294099 MEDLINE
DOCUMENT NUMBER: 99294099 PubMed ID: 10367679
TITLE: BR96 sFv-PE40 immunotoxin: nonclinical safety assessment.
AUTHOR: Haggerty H G; Warner W A; Comereski C R; Peden W M; Mezza L E; Damle B D; Siegall C B; Davidson T J
CORPORATE SOURCE: Department of Drug Safety Evaluation, Bristol-Myers Squibb, Syracuse, New York 13221, USA.. haggerth@bms.com
SOURCE: TOXICOLOGIC PATHOLOGY, (1999 Jan-Feb) 27 (1) 87-94. Ref: 17
Journal code: TOY; 7905907. ISSN: 0192-6233.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990806
Last Updated on STN: 19990806
Entered Medline: 19990729

AB BR96 sFv-PE40, a recombinant DNA-derived fusion **protein** composed of the heavy- and light-chain variable region domains of the monoclonal antibody BR96 and the translocation and catalytic domains of **Pseudomonas exotoxin A**, is being developed for the treatment of solid tumors expressing cell surface Lewis(y)-related antigens. Single- and **repeat**-dose

intravenous toxicity studies in rats and dogs and a comparative ex vivo tissue-binding study with rat, dog, and human tissues were conducted to assess the toxicity of BR96 sFv-PE40 and to estimate a safe starting dose in humans. Additional studies were performed to investigate the prevention of pulmonary vascular-leak syndrome, the dose-limiting toxicity of BR96 sFv-PE40 in rats, and the immunogenicity of BR96 sFv-PE40. In single-dose studies in rats, the vascular leak appeared to be primarily confined to the lungs; however, with a **repeat**-dose regimen (every other day for 5 doses) other organs including the brain and heart were involved at lethal doses (12-15 mg/m2 cumulative). Single doses of 1.8 mg/m2 and a cumulative 3.8 mg/m2 dose (0.75 mg/m2, every other day for 5 doses) were generally well tolerated in rats. These doses are significantly greater than doses required to cure rodents bearing human tumor xenografts. In dogs, the major target organ following single or **repeated** doses (every 3 days for 5 doses) was the pancreas. Morphologic changes in the exocrine pancreas ranged from atrophy with single-cell necrosis to diffuse acinar necrosis. After a 1-mo dose-free observation period, no residual pancreatic toxicity was observed in dogs given single doses up to 6.0 mg/m2 or 5 doses of 2.4 mg/m2 (12 mg/m2 cumulative). No significant pancreatic toxicity was observed at doses <0.6 mg/m2 in high Lewis(y)-expressing dogs. Assessment of trypsinlike immunoreactivity was useful in monitoring changes in pancreatic function. The immunogenicity of BR96 sFv-PE40 could be inhibited by combined treatment with an immunosuppressant in dogs, thus maintaining exposure to BR96 sFv-PE40.

L17 ANSWER 9 OF 28 TOXLIT
ACCESSION NUMBER: 1998:180850 TOXLIT
DOCUMENT NUMBER: CA-130-192473D
TITLE: Renaturation and detection cytobiological activity of recombinant receptor-binding **protein** of **exotoxin A** of **Pseudomonas** aeruginosa.
AUTHOR: Liu X; Ma C; Guo X; Zhu P; Wang J; Zhen Y
CORPORATE SOURCE: Mil. Vet. Inst., Univ. Agric. Anim. Sci., Changchun
SOURCE: Zhongguo Shouyi Xuebao, (1998). Vol. 18, No. 5, pp. 469-472.
CODEN: ZSXUF5. ISSN. 1005-4545.
PUB. COUNTRY: CHINA
DOCUMENT TYPE: Journal; Journal Article
FILE SEGMENT: CA
LANGUAGE: Chinese
OTHER SOURCE: CA 130:192473
ENTRY MONTH: 199904

AB The purified recombinant receptor-binding **protein** of exotoxin A of P. aeruginosa expressed in E. coli was renatured preliminarily by dild. progressively dialysis the renatured sol. **protein** was used in the expt. of competitive inhibition for cytotoxicity of PEA. 10 .mu.G/L PEA incubated with its sensitive cell line L929, about 36 h later, the cytobiol. pathol. change could be found under microscope, but the cell which incubated PEA and renatured recombinant receptor-binding **protein** of PEA was the same as the cell incubated without PEA, which was still viability, division and proliferation until all the cell was aging and death. It is indicated that the action of cytotoxicity of PEA may be inhibited by the recombinant receptor-binding **protein**

of PEA.

L17 ANSWER 10 OF 28 TOXLIT
ACCESSION NUMBER: 1998:68482 TOXLIT
DOCUMENT NUMBER: CA-128-279348Z
TITLE: Purification of recombinant **protein** containing **receptor-binding domain** of **exotoxin A** of **Pseudomonas** aeruginosa.
AUTHOR: Liu X; Guo X; Ma C; Zhu P; Meng R; Li J
CORPORATE SOURCE: Military Veterinary Inst., Univ. Agriculture and Animal Sci., Changchun
SOURCE: Zhongguo Shouyi Xuebao, (1998). Vol. 18, No. 1, pp. 38-41.
CODEN: ZSXUF5. ISSN. 1005-4545.
PUB. COUNTRY: CHINA
DOCUMENT TYPE: Journal; Journal Article
FILE SEGMENT: CA
LANGUAGE: Chinese
OTHER SOURCE: CA 128:279348
ENTRY MONTH: 199806
AB Expressing plasmid contg. **receptor-binding domain** of exotoxin A of P. aeruginosa (PEA), pET-EAB, was transformed into E. coli BL21(DE3). By inducing of IPTG, a recombinant **protein** contg. **receptor-binding domain** of PEA, named PE34, was expressed. PE34 formed inclusion body in expressed E. coli. The expressed E. coli was lyzed by lysozyme-deoxycholic acid sodium and supersonic. The inclusion body was prepd. by centrifugation and washing with 2 mol/L urea with the purifn. rate of the inclusion body being above 75%. Then it was dissolved by 8 mol/L urea and further purified by Sephacryl S-200 gel filter and DEAE-Sepharose Fast Flow ion-exchange chromatog. The purified PE34 appeared a single band in SDS-PAGE gel with its purifn. rate and recovery rate being 95.8% and 24.5%.

L17 ANSWER 11 OF 28 TOXLIT
ACCESSION NUMBER: 1998:62957 TOXLIT
DOCUMENT NUMBER: CA-128-253489W
TITLE: Cloning of **Pseudomonas exotoxin A** receptor binding subunit gene and its expression in Escherichia coli.
AUTHOR: Guo X; Liu X; Zhu P; Liu Z; Meng R; Ma C; Feng S
CORPORATE SOURCE: Mil Vet Inst., Univ. Agric. Animal Sci., Changchun
SOURCE: Zhongguo Shouyi Xuebao, (1997). Vol. 17, No. 3, pp. 226-229.
CODEN: ZSXUF5. ISSN. 1005-4545.
PUB. COUNTRY: CHINA
DOCUMENT TYPE: Journal; Journal Article
FILE SEGMENT: CA
LANGUAGE: Chinese
OTHER SOURCE: CA 128:253489
ENTRY MONTH: 199805
AB For the purpose of treatment of **Pseudomonas exotoxin A**-induced diseases, the **receptor-binding domain** and partial membrane domain of the structure gene of **Pseudomonas exotoxin A** (PEA) (a 1,000 bp DNA segment encoding 309 amino acids) was cloned and expressed under the control of the T7 promotor.

Analyzed by SDS-PAGE the mol. wt. of the **protein** expressed was about 34,000, similar to the putative Mr. and was called PE34. Western-blotting showed that FE34 could react with anti-PEA serum indicating that PE34 should be the target **protein**. SDS-PAGE TLC-scanning showed that PE34 accounted for 56% of the total bacteria **protein**.

L17 ANSWER 12 OF 28 TOXLIT
ACCESSION NUMBER: 1996:170046 TOXLIT
DOCUMENT NUMBER: CA-130-232466N
TITLE: Molecularly guided medicine comprised of fusion **protein** of interleukin-2(60)-PE40 and its recombinant preparation.
AUTHOR: Lu S; Zhang M; Li H
SOURCE: (1996). Faming Zhuanli Shenqing Gongkai Shuomingshu PATENT NO. 1119677 04/03/1996 (Medical Biological Technology Inst. Chinese Academy of Medical Sciences).
CODEN: CNXXEV.
PUB. COUNTRY: CHINA
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: Chinese
OTHER SOURCE: CA 130:232466
ENTRY MONTH: 199904
AB Disclosed is a cell-specific medicine comprised of a fusion **protein** contg. IL-2(60), the N-terminal 60 amino acids encompassing the IL-2 **receptor-binding domain**, and PE40, a mutant form of **Pseudomonas exotoxin A** devoid of its native cell recognition and binding domain and is toxic to IL-2 receptor bearing cells. A recombinant expression vector plasmid pZM10 contg. the fusion **protein**-encoding sequence, an Escherichia coli contg. the vector, and a fusion **protein** expressed by the E.coli are also disclosed.

L17 ANSWER 13 OF 28 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96319746 EMBASE
DOCUMENT NUMBER: 1996319746
TITLE: Translocation of full-length Pseudomonas exotoxin from endosomes is driven by ATP hydrolysis but requires prior exposure to acidic pH.
AUTHOR: Taupiac M.-P.; Alami M.; Beaumelle B.
CORPORATE SOURCE: UMR 5539 CNRS, UM II,34095 Montpellier Cedex 05, France
SOURCE: Journal of Biological Chemistry, (1996) 271/42 (26170-26173).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We attached human transferrin to **Pseudomonas exotoxin A** (PE) to specifically localize this toxin to the endosomal compartment and study its translocation from purified endosomes using a cell-free assay. Transferrin was linked to PE via a disulfide bond. Chemical derivatization inactivated the

PE cell-binding domain, and transferrin-PE was found to be endocytosed via the transferrin receptor only. Transferrin was also conjugated to a truncated PE with no **receptor-binding domain** (PE46). After labeling mouse lymphocytes with radiolabeled transferrin-PE or transferrin-PE46 and endosome isolation, selective translocation of the full-sized toxin portion of the conjugate was observed in a cell-free system. This translocation was strictly dependent upon ATP hydrolysis and was not affected when the acidity of the endosome lumen was neutralized using weak bases, protonophores, or bafilomycin A1. Nevertheless, when present during cell labeling, inhibitors of endosome acidification prevented PE from acquiring translocation competence. Similar inhibition was observed when endocytosis was performed in the presence of brefeldin A, a drug known to interfere with the delivery of endocytic tracers to acidic endosomes. Our data indicate that full-length PE can be transferred to the cytosol directly from endosomes during intoxication by PE conjugates and that, although exposure to acidic pH is a prerequisite for translocation, ATP hydrolysis directly provides the energy required for PE translocation.

L17 ANSWER 14 OF 28 TOXLINE
ACCESSION NUMBER: 1995:208271 TOXLINE
DOCUMENT NUMBER: CRISP-95-D01303-12
TITLE: CONJUGATE INDUCED POLYSACCHARIDE ANTIBODIES.
AUTHOR: SZU S C
CORPORATE SOURCE: NICHD, NIH
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT.
CONTRACT NUMBER: Z01HD01303-12
SOURCE: (1994). Crisp Data Base National Institutes Of Health. Award Type: G = Grant
DOCUMENT TYPE: (RESEARCH)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY MONTH: 199507

AB RPROJ/CRISP Evidence is accruing that serum antibodies to surface polysaccharides, including lipopolysaccharides of Gram-negative organisms, confer protective immunity. The Vi has been licensed by the World Health Organization and the FDA as a vaccine for typhoid fever. To overcome the age-related and T-independent properties of this capsular polysaccharide, the Vi was bound to medically-useful **proteins** by several synthetic schemes. A Phase 1 study confirmed the improved immunologic properties of Vi as a conjugate made with the B-subunit of the heat-labile toxin of Escherichia coli or a recombinant **Pseudomonas** aeruginosa exoprotein A. The Vi was bound to the **protein** by a bifunctional hetero linker, SPDP. Attempts were made to bind the Vi to a **protein** with adipic acid dihydrazide. A semisynthetic Vi was prepared by derivatizing pectin, a plant polysaccharide whose **repeat** unit is alpha-D-(1 ->4)GalA, with acetic anhydride. The product was identical to the Vi with the exception that the C-2 of the treated pectin is O- rather -than N- acetylated. The immunologic properties of the di-O-acetylated pectin have been compared to the Vi. The structure of the Vi, especially the interaction of the carboxyl and the O-acetyl on C-2, was investigated by potentiometric titration, circular dichroism and

reaction with a bulky organic reagent. The data fitted the conclusions drawn from construction of a space filling model in which the surface of the Vi was occupied by the acetyls which covered the carboxyls. Clinical studies confirmed the immunogenicity of a conjugate composed of the detoxified LPS of Vibrio cholerae. The serum antibodies are being analyzed the immunoglobulin composition of their vibriocidal activity. A new sero type cholera 0139 causing epidemics in India is under investigation for its LPS structure and possible cross-reactivity with other cholera. Another LPS, that of E. coli 0157, has been purified and bound to the **exotoxin A** of Clostridium welchii C (pig bel toxin). The resultant conjugate was immunogenic in mice and a lot suitable for clinical study is under synthesis with the objective of evaluating its safety, immunogenicity and ultimately, effective in preventing enteritis caused by this pathogens with especial reference to the complication of the hemolytic uremic syndrome.

L17 ANSWER 15 OF 28 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1994-083210 [10] WPIDS
DOC. NO. CPI: C1994-038174
TITLE: Novel receptor-mediated delivery system comprising a cell **receptor - binding domain**, a cytoplasmic translocation domain and a nuclear translocation signal domain - for transporting macromolecules which can function once internalised.
DERWENT CLASS: B04 D16
INVENTOR(S): BARNETT, T R; DAS, R C
PATENT ASSIGNEE(S): (FARB) BAYER CORP; (MILE) MILES INC
COUNTRY COUNT: 26
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9404696	A1	19940303	(199410)*	EN	36
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE					
W: AU CA FI JP KR NO NZ					
AU 9350885	A	19940315	(199428)		
ZA 9306189	A	19950329	(199518)		34
NO 9500726	A	19950418	(199525)		
EP 658210	A1	19950621	(199529)	EN	
R: BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
FI 9500866	A	19950424	(199529)		
NZ 255870	A	19960925	(199644)		
JP 08504565	W	19960521	(199646)		36
AU 674026	B	19961205	(199706)		
IL 106760	A	19991231	(200018)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
[illegible]6	A1	WO 1993-US7945	19930824
[illegible]5	A	AU 1993-50885	19930824
[illegible]9	A	ZA 1993-6189	19930824
[illegible]	A	WO 1993-US7945	19930824
		NO 1995-726	19950224

Searcher : Shears 308-4994

EP 658210	A1	EP 1993-920291	19930824
		WO 1993-US7945	19930824
FI 9500866	A	WO 1993-US7945	19930824
		FI 1995-866	19950224
NZ 255870	A	NZ 1993-255870	19930824
		WO 1993-US7945	19930824
JP 08504565	W	WO 1993-US7945	19930824
		JP 1994-506592	19930824
AU 674026	B	AU 1993-50885	19930824
IL 106760	A	IL 1993-106760	19930822

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9350885	A	Based on	WO 9404696
EP 658210	A1	Based on	WO 9404696
NZ 255870	A	Based on	WO 9404696
JP 08504565	W	Based on	WO 9404696
AU 674026	B	Previous Publ.	AU 9350885
		Based on	WO 9404696

PRIORITY APPLN. INFO: US 1992-935074 19920825
AN 1994-083210 [10] WPIDS
AB WO 9404696 A UPAB: 19971030
A novel compsn. (I) comprises a **polypeptide** which contains a **receptor-binding domain**, a lytoplasmic translocation domain, a nuclear translocation domain and a means for connecting a selected macromol to the **polypeptide**. Also claimed is a method for inserting an exogenous macromol into a target cell nucleus comprising admin. (I) to target cells, incubating the cells and determining transfer by an assay.

In (I) the **receptor binding domain** is pref. a toxin-derived ligand for a specific cell receptor, e.g. diphtheria toxin or **Pseudomonas exotoxin A** (PEA). The cytoplasmic translocation domain is derived from PEA. The nuclear translocation signal domain is a SV40, yeast alpha-2 or a GAL-4 nucleic acid sequence. The macromol is pref. a nucleotide, oligopeptide, **polypeptide**, **protein**, nucleic acid encoding factor VIII, alpha-1-antitrypsin, a **polypeptide** which is a regulator of gene expression or B-galactosidase. A polycationic macromol, e.g. poly-L-lysine, poly-D-lysine, poly NTS, ornitrine, putrescine, a histone, GAL4, a homeobox domain, spermidine or spermine are used to correct the macromol to the nuclear translocation domain.

USE - (I) provides a novel receptor-mediated delivery system which can transport functional macromolecules that will act once internalised into the nucleus.

Dwg.5d/12

L17 ANSWER 16 OF 28 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 94228535 MEDLINE
DOCUMENT NUMBER: 94228535 PubMed ID: 8174121
TITLE: Targeted therapy with immunotoxins in a nude rat model for leptomeningeal growth of human small cell lung cancer.
AUTHOR: Myklebust A T; Godal A; Fodstad O

CORPORATE SOURCE: Department of Tumor Biology, Norwegian Radium Hospital, Oslo.
SOURCE: CANCER RESEARCH, (1994 Apr 15) 54 (8) 2146-50.
Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940620
Last Updated on STN: 19970203
Entered Medline: 19940606

AB Metastasis to the central nervous system in patients with small cell lung cancer is not uncommon, and a fraction of the cases have leptomeningeal disease for which no effective therapy is available. To establish an experimental model for evaluation of new therapeutic approaches for such tumor lesions, 1 x 10(6) human H-146 cells were injected directly into the cerebrospinal fluid in the cisterna magna of nude rats. Small, superficial leptomeningeal tumors developed, consistently resulting in symptoms of central nervous system involvement after a mean latency of 20 days. The model was used to study the efficacy of intrathecal targeted therapy with immunotoxins. The monoclonal anti-carcinoma antibodies MOC-31 and NrLu10 and the growth factor transferrin were conjugated to **Pseudomonas exotoxin A** (PE), and 1 day after tumor cell inoculation instilled in the cisterna magna as a single bolus dose of 1.5 micrograms. The antibody conjugates, which were highly cytotoxic to target cells in a **protein** synthesis inhibition assay in vitro, increased the symptom-free latency by 35-46%. PE had no effect, reflecting a lower in vitro cytotoxicity and possibly also a down-regulation of transferrin-receptor expression in the meningeal H-146 tumors. Delayed or **repeated** treatment with MOC-31-PE was less effective than day 1 administration, whereas the addition of 10% glycerol to the injection solution increased the symptom-free period to 72%. The efficacy of MOC-31-PE is superior to reported effects obtained in similar models with other therapies, and the results support the development of this immunotoxin towards clinical evaluation in small cell lung cancer patients with leptomeningeal carcinomatosis.

L17 ANSWER 17 OF 28 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 93155177 MEDLINE
DOCUMENT NUMBER: 93155177 PubMed ID: 8429009
TITLE: Residues 1-254 of anthrax toxin lethal factor are sufficient to cause cellular uptake of fused **polypeptides**.
AUTHOR: Arora N; Leppla S H
CORPORATE SOURCE: Laboratory of Microbial Ecology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Feb 15) 268 (5) 3334-41.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930326
Last Updated on STN: 19970203
Entered Medline: 19930309

AB Anthrax lethal toxin is a complex of protective antigen (PA, 735 amino acids) and lethal factor (LF, 776 amino acids) that lyses certain eukaryotic cells. LF interacts with PA to gain access to the cytosol to assert its toxicity. The internalization of LF requires that PA bind to a specific membrane receptor and be cleaved by a cell-surface protease (probably furin), so as to expose a site on PA to which LF binds with high affinity. To localize LF functional domains, amino, carboxyl, and internal deletions of LF were made. Toxicity was eliminated by deletion of 40 and 47 residues from the amino and carboxyl termini, respectively. Similarly, deleting the first of the four imperfect **repeats** of 19 amino acids located at residues 308-383 made LF non-toxic, showing that this region is also essential for activity. To identify the minimum region of LF which is required for binding to PA, varying amino-terminal portions of LF were fused to the ADP-ribosylation domain of **Pseudomonas exotoxin A.**
Fusion **proteins** containing residues 1-254 of LF were toxic when administered with PA, while those having only residues 1-198 of LF were inactive, showing that the PA-binding domain of LF lies within residues 1-254.

L17 ANSWER 18 OF 28 MEDLINE
ACCESSION NUMBER: 94031443 MEDLINE
DOCUMENT NUMBER: 94031443 PubMed ID: 8105849
TITLE: Recombinant fusion toxins--a new class of targeted biologic therapeutics.
AUTHOR: Woodworth T G; Nichols J C
CORPORATE SOURCE: Seragen, Inc., Hopkinton, MA 01748.
SOURCE: CANCER TREATMENT AND RESEARCH, (1993) 68 145-60.
Ref: 25
Journal code: AVA; 8008541. ISSN: 0927-3042.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19950206
Entered Medline: 19931222

AB The design and construction of a new class of recombinant therapeutic agents, receptor-specific cytotoxins, has occurred within the last 5 years. Development of a number of receptor-targeted fusion toxins has been based on a detailed understanding of the structure-function relationships of both diphtheria toxin and **Pseudomonas exotoxin A,** and availability of the nucleic acid sequences of each structural gene. A variety of fusion toxins in which the native **receptor-binding domain** of either diphtheria toxin or **Pseudomonas exotoxin A** has been genetically replaced with either a **polypeptide** hormone or growth factor have been constructed. These fusion toxins selectively intoxicate receptor-bearing cells in

vitro and are active in a variety of animal model systems. DAB486IL-2, and IL-2 receptor targeted cytotoxin, is the first fusion toxin to be evaluated in patients. Phase I/II clinical trials have been performed in refractory leukemia/lymphoma, severe rheumatoid arthritis, and Type 1 diabetes. DAB486IL-2 has been administered to more than 200 patients, has been well tolerated, and has shown encouraging signs of potential efficacy in all three clinical indications. Thus, DAB486IL-2 represents a new class of targeted biological therapeutic response modifiers whose mode of action is based on selective elimination of target cells.

L17 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1994:63087 BIOSIS
DOCUMENT NUMBER: PREV199497076087
TITLE: Development of derivatives of **exotoxin A** of **Pseudomonas** aeruginosa which contain either the fragment of **protein** A of Staphylococcus of interleukin 2 and study on the stability of these **proteins** in E. coli cells.
AUTHOR(S): Zhdanovskii, A. G.; Zhdanovskaya, N. V.; Yankovskii, N. K.; Debabov, V. V.
CORPORATE SOURCE: Res. Inst. Genet. Sel. Ind. Microbiol., Moscow 113545 Russia
SOURCE: Biotekhnologiya, (1993) Vol. 0, No. 6, pp. 15-20. ISSN: 0234-2758.
DOCUMENT TYPE: Article
LANGUAGE: Russian
SUMMARY LANGUAGE: Russian; English
AB Derivatives of exotoxin A lacking the **receptor-binding domain** are non toxic for eukaryotic cells, However such derivatives can be used as the catalytic components of immunotoxins. To make such immunotoxins, exotoxin A derivatives should be connected to **proteins** which recognize specific receptors of eukaryotic cells. To create recombinant immunotoxins hybrid **proteins** were made from gene fusions between fragments of exoioxin A and fragments of staphylococcus **protein** A or interleukin 2. Analysis of these **proteins** has shown that with one exception, all were stable in E. coli.

L17 ANSWER 20 OF 28 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 92225596 MEDLINE
DOCUMENT NUMBER: 92225596 PubMed ID: 1563771
TITLE: Safety, immunogenicity, and efficacy of a Plasmodium falciparum vaccine comprising a circumsporozoite **protein repeat** region **peptide** conjugated to Pseudomonas aeruginosa toxin A.
AUTHOR: Fries L F; Gordon D M; Schneider I; Beier J C; Long G W; Gross M; Que J U; Cryz S J; Sadoff J C
CORPORATE SOURCE: Center for Immunization Research, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205.
CONTRACT NUMBER: R22-AI-29000 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1992 May) 60 (5) 1834-9. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920607
Last Updated on STN: 19980206
Entered Medline: 19920519

AB Twenty-one malaria-naive volunteers were immunized with a vaccine consisting of a 22-kDa recombinant **peptide** (R32LR), derived from the **repeat** region of Plasmodium falciparum circumsporozoite (CS) **protein**, covalently coupled to detoxified Pseudomonas aeruginosa toxin A. Nineteen volunteers received a second dose of vaccine at 8 weeks, and eighteen received a third dose at 8 to 12 months. The vaccine was well tolerated, with only one volunteer developing local discomfort and induration at the site of injection which limited function for 48 h. The geometric mean anti-CS immunoglobulin G antibody concentration 2 weeks after the second dose of vaccine was 10.6 micrograms/ml (standard deviation = 3.0 micrograms/ml). Eleven volunteers (52%) developed anti-CS antibody levels of greater than 9.8 micrograms/ml, the level measured in the one volunteer protected against P. falciparum challenge after immunization with the alum-adjuvanted recombinant **protein** R32tet32 in a prior study. Three separate experimental challenges were conducted with 10 volunteers 2 to 4 weeks after the third dose of vaccine. The four best responders, on the basis of antibody levels (6 to 26 micrograms/ml), were challenged with two infected-mosquito bites, but only one of four immunized volunteers and one of three malaria-naive controls became parasitemic. In a second challenge study using five infected-mosquito bites as the challenge dose, three of three malaria-naive control volunteers and two of three immunized volunteers developed malaria. The third vaccine was apparently completely protected. In the third and last challenge, three of three controls and five of five vaccinees became infected. Sera obtained on the days of challenge inhibited sporozoite invasion of hepatocytes variably in vitro (range, 45 to 90% inhibition), but the degree of inhibition did not correlate with protection. Although antibody against the CS **repeat** region may protect some individuals against experimental challenge, this protection cannot be predicted from antibody levels by current in vitro assays. The functionality and fine specificity of anti-CS antibody are probably critical determinants.

L17 ANSWER 21 OF 28 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 92192793 MEDLINE
DOCUMENT NUMBER: 92192793 PubMed ID: 1548056
TITLE: Lymphoproliferative activity of **Pseudomonas exotoxin A** is dependent on intracellular processing and is associated with the carboxyl-terminal portion.
AUTHOR: Legaard P K; LeGrand R D; Misfeldt M L
CORPORATE SOURCE: Department of Molecular Microbiology and Immunology, University of Missouri-Columbia, School of Medicine 65212.
CONTRACT NUMBER: AI-19359 (NIAID)
T32-AI07279 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1992 Apr) 60 (4) 1273-8.
Journal code: GO7; 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920509
Last Updated on STN: 20000303
Entered Medline: 19920423

AB **Pseudomonas** aeruginosa **exotoxin A** (PE) represents a microbial superantigen that requires processing by accessory cells in order to induce the proliferation of V beta 8-bearing murine T lymphocytes. In this study, we have observed that PE requires intracellular processing by a protease in order to induce lymphoproliferation. Pepstatin A, an inhibitor of acid proteases, inhibited PE-induced lymphoproliferation, whereas leupeptin, an inhibitor of serine and thiol proteases, had no effect on PE-induced lymphoproliferation. A number of mutant forms of PE were examined for their ability to induce lymphoproliferation. The mutant form which lacks amino acids 5 to 224 of the **receptor -binding domain**, PE43, was capable of inducing murine thymocytes to proliferate in the presence of accessory cells. However, neither PEgly276, a mutant toxin which undergoes a different intracellular processing pattern than wild-type PE, nor PE589, a mutant toxin which lacks amino acids 590 to 613 at the carboxyl terminus, was able to induce thymocyte proliferation. In addition, the lymphoproliferation induced by the PE43 mutant form of PE could also be inhibited by pepstatin A. Therefore, our data indicate that intracellular processing by a proteolytic enzyme which is inhibited by pepstatin A is critical for PE-induced lymphoproliferation. Furthermore, the lymphoproliferative activity of PE is associated with the carboxyl-terminal portion of PE.

L17 ANSWER 22 OF 28 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 91373357 MEDLINE
DOCUMENT NUMBER: 91373357 PubMed ID: 1910044
TITLE: Increased cytotoxic activity of Pseudomonas exotoxin and two chimeric toxins ending in KDEL.
AUTHOR: Seetharam S; Chaudhary V K; FitzGerald D; Pastan I
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Sep 15) 266 (26) 17376-81.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19911108
Last Updated on STN: 19990129
Entered Medline: 19911021

AB Pseudomonas exotoxin (PE) is a 66,000 molecular weight **protein** secreted by Pseudomonas aeruginosa. PE is made three domains, and PE40 is a form of PE which lacks dom acids 1-252) and has very low cytotoxicity because it c target cells. The sequence Arg-Glu-Asp-Leu-Lys (REDLK) carboxyl terminus of Pseudomonas exotoxin has been show

important for its cytotoxic activity (Chaudhary, V. K., Jinno, Y., FitzGerald, D. J., and Pastan, I. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 308-312). In this study, we tested the effect of altering the carboxyl sequence of PE from REDLK to the characteristic endoplasmic reticulum retention sequence, KDEL, or to KDEL **repeated** three times (KDEL)3. We also made similar changes at the carboxyl terminus of two chimeric toxins in which domain I of PE (amino acids 1-252) was either replaced with transforming growth factor alpha (TGF alpha) to make TGF alpha-PE40 or with a single chain antibody (anti-Tac) reacting with the human interleukin 2 receptor to make anti-Tac(Fv)-PE40. Statistical analyses of our results demonstrate that PE and its derivatives ending in KDEL or (KDEL)3 are significantly more active than PE or derivatives ending in REDLK. We have also found that brefeldin A, which is known to perturb the endoplasmic reticulum, inhibits the cytotoxic action of PE. Our results suggest that the altered carboxyl terminus may enable the toxin to interact more efficiently with a cellular component involved in translocation of the toxin to the cytosol.

L17 ANSWER 23 OF 28 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 10
ACCESSION NUMBER: 91155751 EMBASE
DOCUMENT NUMBER: 1991155751
TITLE: Functional analysis of **exotoxin A** -related **protein** of **Pseudomonas** aeruginosa lacking residues 225-412.
AUTHOR: Guidi-Rontani C.
CORPORATE SOURCE: Unite des Antigenes Bacteriens, URA-CNRS 557, Institut Pasteur,75015 Paris, France
SOURCE: FEMS Microbiology Letters, (1991) 80/1 (103-109). ISSN: 0378-1097 CODEN: FMLED7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The crystal structure of the **exotoxin A** (ETA) of **Pseudomonas** aeruginosa showed that this **protein** is folded into three distinct domains. Domain I (Ia and Ib), the amino-terminal domain, is the **receptor-binding domain** of ETA and domain III, the carboxy-terminal domain, is responsible for the ADP-ribosyl transferase activity of the toxin. To elucidate the function(s) of domains 1b and II in the intoxication process and to define the region of the domain III necessary for ADP-ribosylating activity, a defined deletion in the structural gene of P. aeruginosa ETA encompassing residues 225-412 was constructed and an ETA-related product DeID, (from which all of domains II and Ib were deleted) was expressed. The ETA-related **protein** did not penetrate sensitive cells, but retained the same specific activity to ADP-ribosylate elongation factor-2 as wild-typed toxin. This suggests that domain II is necessary to allow toxin internalization by sensitive cells and that the absence of domain Ib does not interfere with enzymatic activity. The domain strictly involved in ADP-ribosylation activity encompasses residues 412-613.

L17 ANSWER 24 OF 28 MEDLINE
ACCESSION NUMBER: 91309859 MEDLINE

DOCUMENT NUMBER: 91309859 PubMed ID: 1906825
TITLE: Functional analysis of **exotoxin A** -related **protein** of **Pseudomonas** aeruginosa lacking residues 225-412.
AUTHOR: Guidi-Rontani C
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA.
CONTRACT NUMBER: AI22021 (NIAID)
AI22848 (NIAID)
SOURCE: FEMS MICROBIOLOGY LETTERS, (1991 May 1) 64 (1) 103-9.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910913
Last Updated on STN: 19910913
Entered Medline: 19910826

AB The crystal structure of the **exotoxin A** (ETA) of **Pseudomonas** aeruginosa showed that this **protein** is folded into three distinct domains. Domain I (Ia and Ib), the amino-terminal domain, is the **receptor-binding domain** of ETA and domain III, the carboxy-terminal domain, is responsible for the ADP-ribosyl transferase activity of the toxin. To elucidate the function(s) of domains 1b and II in the intoxication process and to define the region of the domain III necessary for ADP-ribosylating activity, a defined deletion in the structural gene of P. aeruginosa ETA encompassing residues 225-412 was constructed and an ETA-related product DeID, (from which all of domains II and Ib were deleted) was expressed. The ETA-related **protein** did not penetrate sensitive cells, but retained the same specific activity to ADP-ribosylate elongation factor-2 as wild-type toxin. This suggests that domain II is necessary to allow toxin internalization by sensitive cells and that the absence of domain Ib does not interfere with enzymic activity. The domain strictly involved in ADP-ribosylation activity encompasses residues 412-613.

L17 ANSWER 25 OF 28 TOXLINE
ACCESSION NUMBER: 1991:201077 TOXLINE
DOCUMENT NUMBER: BIOSIS-91-26519
TITLE: EPITOPE MAPPING ANALYSIS OF THE **RECEPTOR-BINDING DOMAIN** OF **PSEUDOMONAS**-AERUGINOSA **EXOTOXIN A** SELECTION OF MONOCLONAL ANTIBODIES USEFUL IN THE PREPARATION OF RECEPTOR-BINDING ANTI-IDIOTYPIC ANTIBODIES.
AUTHOR: ROLF J M; BERKI T; LANG A B; EIDELS L
SOURCE: (1991). Vol. 91, pp. 73. 91ST GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY 1991, DALLAS, TEXAS, USA, MAY 5-9, 1991. ABSTR GEN MEET AM SOC MICROBIOL.
CODEN: AGMME8.
FILE SEGMENT: BIOSIS
LANGUAGE: English
ENTRY MONTH: 199110

L17 ANSWER 26 OF 28 MEDLINE
ACCESSION NUMBER: 90115863 MEDLINE
DOCUMENT NUMBER: 90115863 PubMed ID: 2104981
TITLE: Pseudomonas exotoxin contains a specific sequence at the carboxyl terminus that is required for cytotoxicity.
AUTHOR: Chaudhary V K; Jinno Y; FitzGerald D; Pastan I
CORPORATE SOURCE: Division of Cancer Biology and Diagnosis, National Cancer Institute, Bethesda, MD 20892.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Jan) 87 (1) 308-12.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199002
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19970203
Entered Medline: 19900209

AB Pseudomonas exotoxin (PE), a single-chain **polypeptide** toxin of 613 amino acids, consists of three functional domains: an amino-terminal **receptor-binding domain**, a middle translocation domain, and a carboxyl-terminal ADP-ribosylation domain. Deletion of as few as 2 or as many as 11 amino acids from the carboxyl terminus of PE does not affect ADP-ribosylation activity but produces noncytotoxic molecules. Deletions and substitutions between positions 602 and 611 of PE show that the last 5 amino acids of PE are very important for its cytotoxic action. The carboxyl-terminal sequence of PE is Arg-Glu-Asp-Leu-Lys. Mutational analysis indicates that a basic amino acid at 609, acidic amino acids at 610 and 611, and a leucine at 612 are required for full cytotoxic activity. Lysine at 613 can be deleted or replaced with arginine but not with several other amino acids. Mutant toxins are able to bind normally to target Swiss mouse 3T3 cells and are internalized by endocytosis, but apparently they do not penetrate into the cytosol. A PE molecule that ends with Lys-Asp-Glu-Leu, which is a well defined endoplasmic reticulum retention sequence [Munro, S. and Pelham, R. B. (1987) Cell 48, 899-907], is fully cytotoxic, suggesting that a common factor may be involved in intoxication of cells by PE and retention of **proteins** in the lumen of the endoplasmic reticulum. Sequences similar to those at the carboxyl end of PE are also found at the end of Cholera toxin A chain and Escherichia coli heat-labile toxin A chain.

L17 ANSWER 27 OF 28 MEDLINE
ACCESSION NUMBER: 87057009 MEDLINE
DOCUMENT NUMBER: 87057009 PubMed ID: 2430945
TITLE: Analysis of transcription of the **exotoxin A** gene of **Pseudomonas** aeruginosa.
AUTHOR: Grant C C; Vasil M L
CONTRACT NUMBER: AI 15940 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1986 Dec) 168 (3) 1112-9.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198701
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19870114

AB Analysis of RNA isolated from **Pseudomonas** aeruginosa PA103 and PAKS grown under Fe2+-limiting (0.08 microgram/ml) and Fe2+-sufficient (10 micrograms/ml) conditions demonstrated that **exotoxin A** (ETA) expression is regulated by Fe2+ at the level of transcription. S1 nuclease mapping revealed two 5' termini of the tox transcript, 89 base pairs (bp) (S1A) and 62 bp (S1B) 5' to the ETA initiation codon. There appeared to be no consensus promoter sequence for either tox transcript. An 8-bp direct **repeat** was found 5' to the start of transcript S1A. Transcript S1B mapped 8 bp upstream of a dodecamer sequence conserved between the ETA and phospholipase C genes of P. aeruginosa. Multicopy plasmids in which the expression of ETA is directed from the Escherichia coli trp promoter (ptrpETA-RSF1010) or the tox promoter (pCMtox) were constructed and mobilized into a Tox-P. aeruginosa strain, WR5. WR5 synthesized and secreted high levels of ETA when it was expressed from the E. coli trp promoter; however, the synthesis of ETA from its own promoter in this strain was very low. These and other data suggest that the expression of ETA is under a positive control mechanism. A fusion of the ETA promoter fragment to lacZ was constructed. Use of this fusion plasmid revealed that this DNA fragment directed the synthesis of beta-galactosidase in E. coli at very low levels and that the synthesis of beta-galactosidase from this fusion in E. coli was not regulated by Fe2+.

L17 ANSWER 28 OF 28 MEDLINE
ACCESSION NUMBER: 85230628 MEDLINE
DOCUMENT NUMBER: 85230628 PubMed ID: 3924608
TITLE: Enzyme-linked immunosorbent assay for detection of antibodies to Pseudomonas aeruginosa exoproteins.
AUTHOR: Granstrom M; Wretlind B; Markman B; Pavlovskis O R; Vasil M L
CONTRACT NUMBER: AI 15940 (NIAID)
SOURCE: EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY, (1985 Apr) 4 (2) 197-200.
Journal code: EMY; 8219582. ISSN: 0722-2211.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198508
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 20000303
Entered Medline: 19850814

AB Enzyme-linked immunosorbent assays were developed with four purified **Pseudomonas** aeruginosa extracellular **proteins** (**exotoxin A**, elastase, alkaline protease, and phospholipase C) to determine antibody levels in sera from healthy subjects and the serological response in patients colonized or infected with **Pseudomonas** aeruginosa. Five of 39 burn patients with wounds colonized by **Pseudomonas** aeruginosa

had elevated antibody titers to alkaline protease. Response to the other antigens was found in only a few patients. **Pseudomonas** aeruginosa infections (septicemia, osteitis, pneumonia etc.) resulted in increased antibody levels to **exotoxin A** or phospholipase C in 15 of 22 patients. These findings suggest that **repeated** determinations of antibodies to **Pseudomonas** aeruginosa **exotoxin A** and phospholipase C might be used to monitor therapy in certain patients with osteitis and other deep **Pseudomonas** infections.

FILE 'REGISTRY' ENTERED AT 15:05:02 ON 13 NOV 2001
L18 138 SEA ABB=ON PLU=ON EHWSYGLRPG|LIGICVAVTVAI|MHLIPHWIPLVAS LGLLAGGSSAL/SQSP

Seq IDs 1-3

FILE 'CAPLUS' ENTERED AT 15:05:50 ON 13 NOV 2001
L19 59 SEA ABB=ON PLU=ON L18
L20 1 SEA ABB=ON PLU=ON L19 AND L9
L21 0 SEA ABB=ON PLU=ON L20 NOT L13

FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 15:07:54 ON 13 NOV 2001
L22 6727 S HWANG J?/AU
L23 8973 S HSU C?/AU
L24 1350 S TING C?/AU
L25 13 S L22 AND L23 AND L24
L26 38 S L22 AND (L23 OR L24)
L27 15 S L23 AND L24
L28 74 S (L22 OR L23 OR L24) AND L9
L29 59 S (L22 OR L23 OR L24) AND L10
L30 88 S L25 OR L26 OR L27 OR L29
L31 34 DUP REM L30 (54 DUPLICATES REMOVED)

- Author(s)

L31 ANSWER 1 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:261138 CAPLUS
DOCUMENT NUMBER: 134:294520
TITLE: Method for making fusion **protein** vaccines using repeat immunogens and receptor binding domain of a Pseudomonas exotoxin
INVENTOR(S): **Hwang, Jaulang; Hsu, Chia-Tse ; Ting, Chun-Jen**
PATENT ASSIGNEE(S): Academia Sinica, Taiwan
SOURCE: Eur. Pat. Appl., 15 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1090994	A2	20010411	EP 2000-304253	20000519
EP 1090994	A3	20010718		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
PRIORITY APPLN. INFO.: US 1999-412558 A 19991005
AB The invention provides a method for making **protein**-based vaccines using a receptor binding domain of a **Pseudomonas exotoxin A** or a functional variant thereof, and at

least two copies of a **peptide** sequence. The invention is based on the discovery of a new means of generating an immune response to a **peptide** antigen by concatenating the **peptide** and fusing the concatemer to a receptor binding domain of a Pseudomonas exotoxin. Such a fusion **protein** elicits antigen-specific antibodies in a variety of mammals, with little or no toxicity obsd. In particular, the invention provides two new multimeric vaccines, against **vaccinia** virus and against **gonadotropin releasing hormone** , resp.

L31 ANSWER 2 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:152357 CAPLUS
DOCUMENT NUMBER: 134:192236
TITLE: Pseudomonas fusion **protein** vaccines
INVENTOR(S): **Hwang, Jaulang**; Shang, Huey-fang; Chen, Tzong-yueh
PATENT ASSIGNEE(S): Academia Sinica, Taiwan
SOURCE: Eur. Pat. Appl., 14 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1078988	A1	20010228	EP 1999-306862	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001078765	A2	20010327	JP 1999-243264	19990830

PRIORITY APPLN. INFO.: EP 1999-306862 A 19990827

AB A fusion **protein** suitable as a vaccine is provided that contains at least three Pseudomonas antigens or antigenic fragments. These polypepitde moieties comprise: (1) a receptor binding domain of **Pseudomonas exotoxin A** functional variant thereof; (2) a membrane translocation domain of **Pseudomonas exotoxin A** or functional variant thereof; (3) a **Pseudomonas** lipoprotein I or functional variant thereof, or antigenic fragment of a **Pseudomonas** lipoprotein I or functional variant thereof; and (4) an antigenic C-terminal fragment of a **Pseudomonas** porin **protein** F or functional variant thereof. Such a fusion **protein** was constructed comprising (His)6-PE1-405-OprI19-83-OprF24-350 (I): i.e., a histidine affinity tag attached to residues 1-405 of the **Pseudomonas** aeruginosa **exotoxin A**, which is then attached to residues 19-83 of the **Pseudomonas** lipoprotein I, and finally residues 24-350 of **Pseudomonas** porin **protein** F. I induces higher levels of anti-PE antibodies than an immunogen including PE alone, and the antibodies are capable of neutralizing the cytotoxicity of PE on NIH3T3 cells. I also affords significantly higher protection (80%) against challenge with PE-hyper-producing strain PA103 than OprF alone (40%).

REFERENCE COUNT: 2
REFERENCE(S):
(1) Behringwerke Ag; EP 0717106 A 1996 CAPLUS
(2) The Government Of The United States; WO 9902713 A 1999 CAPLUS

L31 ANSWER 3 OF 34 MEDLINE
ACCESSION NUMBER: 2001574760 IN-PROCESS
DOCUMENT NUMBER: 21538895 PubMed ID: 11546768
TITLE: 26 S Proteasome-mediated Degradation of Topoisomerase II Cleavable Complexes.
AUTHOR: Mao Y; Desai S D; **Ting C Y; Hwang J** ; Liu L F
CORPORATE SOURCE: Department of Pharmacology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854-5635 and the Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Nov 2) 276 (44) 40652-8.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20011030
Last Updated on STN: 20011030

AB DNA topoisomerase II (TOP2) cleavable complexes represent an unusual type of DNA damage characterized by reversible TOP2-DNA cross-links and DNA double strand breaks. Many antitumor drugs and physiological stresses are known to induce TOP2 cleavable complexes leading to apoptotic cell death and genomic instability. However, the molecular mechanism(s) for repair of TOP2 cleavable complexes remains unclear. In the current studies, we show that TOP2 cleavable complexes induced by the prototypic TOP2 poison VM-26 are proteolytically degraded by the ubiquitin/26 S proteasome pathway. Surprisingly the TOP2beta isozyme is preferentially degraded over TOP2alpha isozyme. In addition, transcription inhibitors such as 5,6-dichlorobenzimidazole riboside and camptothecin can substantially block VM-26-induced TOP2beta degradation. These results are consistent with a model in which the repair of TOP2beta cleavable complexes may involve transcription-dependent proteolysis of TOP2beta to reveal the protein-concealed double strand breaks.

L31 ANSWER 4 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
ACCESSION NUMBER: 2001:711570 CAPLUS
TITLE: Photoluminescence and electroluminescence characteristics of new disubstituted polyacetylenes
AUTHOR(S): **Ting, Ching Hua; Hsu, Chain Shu**
CORPORATE SOURCE: Department of Applied Chemistry, National Chiao Tung University, Hsinchu, 30050, Taiwan
SOURCE: Jpn. J. Appl. Phys., Part 1 (2001), 40(9A), 5342-5345
CODEN: JAPNDE; ISSN: 0021-4922
PUBLISHER: Japan Society of Applied Physics
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three di-substituted acetylenes in the tolane structure, 4-(trans-4-pentylcyclohexyl)-3',4'-difluorotolane (1M), 4-(trans-4-heptylcyclohexyl)-4'-fluorotolane (2M), and 4-(4-pentylphenyl)-4'-fluorotolane (3M), were polymd. in the

presence of TaCl5-based catalyst. The wt.-av. mol. wts. Mw of the polymers were high than 4 .times. 105. Photoluminescence (PL) and electroluminescence (EL) of the three polymers made as single-layer device on indium-tin oxide glass (ITO), ITO/polymer/Al, have been comprehensively studied. By changing the structural conditions of polymer, such as introducing the fluorine atom or a long carbon chain to the end group of polymer side chains, the luminescence is clearly enhanced.

REFERENCE COUNT: 17
REFERENCE(S):
(1) Carter, P; Phys Rev B 1991, V43, P14478 CAPLUS
(2) Hidayat, R; Jpn J Appl Phys 1998, V37, PL180 CAPLUS
(3) Hidayat, R; Synth Met 1999, V101, P210 CAPLUS
(5) Hirohata, M; Jpn J Appl Phys 1997, V36, PL302 CAPLUS
(6) Huang, Y; Thin Solid Films 2000, V363, P146 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 5 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4

ACCESSION NUMBER: 2000:525737 CAPLUS
DOCUMENT NUMBER: 133:236494

TITLE: Vaccination against **gonadotropin-releasing hormone** (**GnRH**) using toxin receptor-binding domain-conjugated **GnRH** repeats

AUTHOR(S): **Hsu, Chia-Tse; Ting, Chun-Yuan ; Ting, Chun-Jen**; Chen, Tzong-Yueh; Lin, Chia-Po; Whang-Peng, Jacqueline; **Hwang, Jaulang**
CORPORATE SOURCE: Graduate Institute of Life Science, National Defense Medical Center, Institute of Molecular Biology, Academia Sinica, Taipei, 11529, Taiwan

SOURCE: Cancer Res. (2000), 60(14), 3701-3705
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A method for the prepn. of an immunogen contg. multiple copies of a self-**peptide** in linear alignment was designed to overcome the difficulty of inducing an immune response to poorly immunogenic **peptide** antigens. DNA fragments encoding multiple repeats of the self-**peptide** were generated by a new technique, termed template-repeated polymerase chain reaction (TR-PCR), which could be subcloned into an expression vector for prodn. of **peptide** repeats as an immunogen. This approach was tested by constructing fusion **proteins** contg. the receptor-binding domain of **Pseudomonas exotoxin A** and multiple copies of the 10-residue sequence of the **peptide** hormone **gonadotropin-releasing hormone** (**GnRH**). Immunization of female rabbits with the immunogen that contained the exotoxin receptor-binding domain and 12 copies of **GnRH** (PEIa-GnRH12) resulted in the generation of high-titer antibodies specific for **GnRH**. Although at equal molar basis of the **GnRH** moiety, the immunogen that contained single copy of **GnRH** (PEIa-GnRH1)

induced low-titer anti-**GnRH** antibodies. These observations suggest that the presence of multiple **peptide** repeats is a key factor in eliciting an immune response. In addn., anti-**GnRH** antibodies effectively neutralized **GnRH** activity in vivo, as demonstrated by the degeneration of the ovaries in the injected rabbits. Because anti-**GnRH** antibody could be functionally analogous to **GnRH** antagonist, which has been used to treat patients with ovarian cancer, vaccination of PEIa-GnRH12 presents a potential therapeutic application for the treatment of **GnRH**-sensitive ovarian cancer.

REFERENCE COUNT: 16

REFERENCE(S):
(1) Baselga, J; Cancer Res 1998, V58, P2825 CAPLUS
(2) Baselga, J; J Clin Oncol 1996, V14, P737 CAPLUS
(3) Baselga, J; J Natl Cancer Inst 1993, V85, P1327 CAPLUS
(4) Conn, P; Fed Proc 1984, V43, P2351 CAPLUS
(5) Eidne, K; Science (Washington DC) 1985, V229, P989 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 6 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5

ACCESSION NUMBER: 2000:404940 CAPLUS
DOCUMENT NUMBER: 133:291656
TITLE: Development of DNA delivery system using **Pseudomonas exotoxin A** and a DNA binding region of human DNA topoisomerase I
AUTHOR(S): Chen, T.-Y.; **Hsu, C.-T.**; Chang, K.-H.; **Ting, C.-Y.**; Whang-Peng, J.; Hui, C.-F.; **Hwang, J.**
CORPORATE SOURCE: Institute of Genetics, School of Life Science, National Yang-Ming University, Taipei, Taiwan
SOURCE: Appl. Microbiol. Biotechnol. (2000), 53(5), 558-567
CODEN: AMBIDG; ISSN: 0175-7598
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Gene therapy is defined as the delivery of a functional gene for expression in somatic tissues with the intent to cure a disease. Thus, highly efficient gene transfer is essential for gene therapy. Receptor-mediated gene delivery can offer high efficiency in gene transfer, but several tech. difficulties need to be solved. In this study, the authors first examd. the DNA binding regions of the human DNA topoisomerase I (Topo I), using agarose gel mobility shift assay, in order to identify sites of noncovalent binding of human DNA Topo I to plasmid DNA. The authors identified four DNA binding regions in human DNA Topo I. They resided in aa 51-200, 271-375, 422-596, and 651-696 of the human DNA Topo I. The authors then used one of the four regions as a DNA binding **protein** fragment in the construction of a DNA delivery vehicle. Based on the known functional property of each **Pseudomonas exotoxin A** (PE) domain and human DNA Topo I, the authors fused the receptor binding and membrane translocation domains of PE with a highly pos. charged DNA binding region of the N-terminal 198 amino acid residues of human DNA Topo I. The resulting recombinant

protein was examd. for DNA binding in vitro and transfer efficiency in cultured cells. The results show that this DNA delivery **protein** is a general DNA delivery vehicle without DNA sequence, topol., and cell-type specificity. The DNA delivery **protein** could be used to target genes of interest into cells for genetic and biochem. studies. Therefore, this technique can potentially be applied to cancer gene therapy.

REFERENCE COUNT: 42

REFERENCE(S):
(1) Allured, V; Proc Natl Acad Sci USA 1986, V83, P1320 CAPLUS
(2) Alsner, J; J Biol Chem 1992, V267, P12408 CAPLUS
(3) Bharti, A; J Biol Chem 1996, V271, P1993 CAPLUS
(4) Champoux, J; DNA topology and its biological effects 1990, P217 CAPLUS
(5) Chen, T; Appl Microbiol Biotechnol 1999, V52, P524 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 7 OF 34 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 2000210512 MEDLINE
DOCUMENT NUMBER: 20210512 PubMed ID: 10746417
TITLE: Surgical repair of postinfarction ventricular septal defect.
AUTHOR: Wang J S; **Hsu C P**; Yu T J; **Hwang J S**; Shiu C T; Lai S T
CORPORATE SOURCE: Department of Surgery, Taipei Veterans General Hospital, Taiwan, ROC.
SOURCE: CHUNG-HUA I HSUEH TSA CHIH [CHINESE MEDICAL JOURNAL], (2000 Mar) 63 (3) 213-9.
Journal code: CHQ; 0005327. ISSN: 0578-1337.
PUB. COUNTRY: China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000427

AB BACKGROUND: Rupture of the interventricular septum complicates 1% to 2% of all acute myocardial infarction patients and its natural course is ominous. The purpose of this study is to present our experience with surgical ventricular septal defect (VSD) repair and examine the possible risk factors and explanations for surgical mortality. METHODS: Fourteen patients underwent repair of postinfarction VSD from 1996 to 1998 at the Taipei Veterans General Hospital. Thirteen patients were in New York Heart Association (NYHA) Functional Class IV and one was in Functional Class III. Eleven patients were in cardiogenic shock with intra-aortic balloon pumps (IABPs) prior to surgery. The operative techniques for VSD repair range from extensive infarctectomy with reconstruction of the septum and the right and left ventricular free walls using single or double patches, to minimal or no infarctectomy with closure of the VSD by excluding the infarcted muscle from the left ventricular cavity and leaving the right ventricle intact. RESULTS: Overall surgical mortality occurred in four patients. All deaths occurred in patients with cardiogenic shock, two with anterior VSD and two with

posterior VSD. Three late survivors had limited exercise tolerance with NYHA Functional Class II to III. Left ventricular function was moderately impaired in most patients with a mean nuclear scan ejection fraction of 0.32. However, all patients were elderly and adapted to their residual symptoms without significant life-style changes. CONCLUSIONS: The surgical mortality for treating patients with postinfarction VSD has decreased with improvements in surgical technique. Rapid diagnosis, appropriate preoperative management and delicate surgical repair improve the overall results and help to attain long-term survival.

L31 ANSWER 8 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1999:726142 CAPLUS
DOCUMENT NUMBER: 132:34441
TITLE: Recombinant **protein** composed of **Pseudomonas exotoxin A**, outer membrane **proteins** I and F as vaccine against P. aeruginosa infection
AUTHOR(S): Chen, T.-Y.; Shang, H.-F.; Chen, T.-L.; Lin, C.-P.; Hui, C.-F.; **Hwang, J.**
CORPORATE SOURCE: Institute of Genetics, School of Life Sciences, National Yang-Ming University, Taipei, 115, Taiwan
SOURCE: Appl. Microbiol. Biotechnol. (1999), 52(4), 524-533
CODEN: AMBIDG; ISSN: 0175-7598
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have constructed a chimeric **protein** composed of the receptor binding and membrane translocation domains of **Pseudomonas exotoxin A** (PE) with the outer membrane **proteins** I and F, together designated as PEIF. The potential of PEIF as a vaccine against Pseudomonas infection was evaluated in BALB/c mice and New Zealand white rabbits. We examd. titers of anti-PE and anti-OprF antibodies, and the ability both to neutralize PE cytotoxicity and to increase opsonophagocytic uptake of Pseudomonas aeruginosa strain PAO1, serogroups 2 and 6. The results showed that PEIF can induce antibodies not only to neutralize the PE cytotoxicity but also to promote the uptake of various strains of P. aeruginosa by murine peritoneal macrophages. In a burned mouse model, PEIF afforded significant protection against infection by the homologous P. aeruginosa strain PAO1, heterologous serogroup 2, and the PE hyperproducing strain PA103. These observations thus indicate that PEIF may be used as a novel vaccine against P. aeruginosa infection.
REFERENCE COUNT: 42
REFERENCE(S):
(4) Chow, J; J Biol Chem 1989, V264, P18818 CAPLUS
(7) Cryz, S; Infect Immun 1984, V43, P795 CAPLUS
(8) Cryz, S; Infect Immun 1986, V52, P161 CAPLUS
(9) Cryz, S; Infect Immun 1987, V55, P1547 CAPLUS
(10) Cryz, S; J Clin Invest 1987, V80, P51 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 9 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8

ACCESSION NUMBER: 1999:641670 CAPLUS
DOCUMENT NUMBER: 131:256069
TITLE: A nontoxic **Pseudomonas exotoxin A** induces active immunity and passive protective antibody against **Pseudomonas exotoxin A** intoxication
AUTHOR(S): Chen, Tzong-Yueh; Lin, Chia Po; Loa, Chien-Chang; Chen, Tso-Ling; Shang, Huey-Fang; **Hwang, Jau Lang**; Hui, Cho-Fat
CORPORATE SOURCE: Institute Genetics, School Life Science, National Yang-Ming Univ., Taipei, 115, Taiwan
SOURCE: J. Biomed. Sci. (Basel) (1999), 6(5), 357-363
CODEN: JBCIEA; ISSN: 1021-7770
PUBLISHER: S. Karger AG
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Pseudomonas exotoxin A** (PE) is one of the most potent cytotoxic agents produced by P. aeruginosa. The authors examd. the possibility of using PE with a deletion of 38 carboxyl-terminal amino acid residues, designated PE(.DELTA.576-613), for active immunization against PE-mediated disease. We 1st examd. the toxic effects of PE and PE(.DELTA.576-613) on 5- and 9-wk-old ICR mice. The results show that the s.c. administration of PE(.DELTA.576-613) at a dose of 250 .mu.g was still nontoxic to 5- and 9-wk-old ICR mice, while native PE was lethal at a dose of 0.5 and 1 .mu.g, resp. PE(.DELTA.576-613) was then used to immunize ICR mice. The min. dose of PE(.DELTA.576-613) that could effectively induce anti-PE antibodies in 5- and 9-wk-old ICR mice was 250 ng. Immunization with 250 ng PE(.DELTA.576-613) failed to protect the immunized mice from a LD of PE. The effective immunization dose of PE(.DELTA.576-613) that could protect mice against a 2 .mu.g PE challenge was 15 .mu.g. Blood sera from PE(.DELTA.576-613)-immunized ICR mice were able to neutralize PE intoxication and effectively protect mice from PE. Thus, PE(.DELTA.576-613) may be used as an alternative route to new PE vaccine development.

REFERENCE COUNT: 41
REFERENCE(S):
(1) Allured, V; Proc Natl Acad Sci USA 1986, V83, P1320 CAPLUS
(4) Chen, S; J Gen Microbiol 1987, V133, P3081 CAPLUS
(5) Chow, J; J Biol Chem 1989, V264, P18818 CAPLUS
(7) Cryz, S; Infect Immun 1984, V43, P795 CAPLUS
(8) Cryz, S; Infect Immun 1986, V52, P161 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 10 OF 34 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000055048 EMBASE
TITLE: Comparison between subxiphoid approach and left thoracotomy in surgical treatment of malignant pericardial effusion - The experience of Taipei veterans general hospital.
AUTHOR: **Hsu C.-P.**; Yu T.-J.; Lai S.-T.; Weng Z.-C.; **Hwang J.-H.**; Shih C.-T.; Wang J.- S.
CORPORATE SOURCE: Dr. C.-P. Hsu, Division of Cardiovascular Surgery, Department of Surgery, Veterans General Hospital

Taipei, No. 201, Shih-Pai Road, Taipei 112, Taiwan, Province of China
SOURCE: Acta Cardiologica Sinica, (1999) 15/2 (73-79).
Refs: 8
ISSN: 1011-6842 CODEN: CKHCE3
COUNTRY: Taiwan, Province of China
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
LANGUAGE: English
SUMMARY LANGUAGE: English; Chinese

AB There are several methods to release pericardial tamponade or pericardial effusion. In this study, we evaluated two different surgical approaches: subxiphoid pericardial drainage and left anterior thoracotomy. From 1/1993 to 5/1998, 22 patients (16 male and 6 female, aged 28-81 years) with malignant pericardial effusion with or without cardiac tamponade were treated with surgical intervention. Among them, 12 patients were treated with the subxiphoid approach and 9 patients with the thoracotomy approach. Another one patient received thoracotomy followed by subxiphoid approach because of recurrent pericardial effusion. The underlying etiology of malignancy for pericardial effusion was similar between the two groups. Symptoms were partially relieved by pericardiocentesis before operation in 7 patients. Though there were no deaths or major complications attributable to surgery itself, 4 patients died of underlying diseases within one month after operation. The overall 30-day mortality was 4/22. To evaluate the effect of surgery, 16 patients were followed up with echocardiography at least 2 weeks after removal of drainage tube. The cumulative effusion-free rate was 100% (10/10) in patients with the subxiphoid approach and 50% (3/6) in patients with the thoracotomy approach (p = 0.063). Compared with left anterior thoracotomy, subxiphoid pericardial drainage seems a more efficient treatment, with low morbidity, for malignant pericardial effusion.

L31 ANSWER 11 OF 34 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-480507 [41] WPIDS
CROSS REFERENCE: 1998-260648 [23]
DOC. NO. NON-CPI: N1998-374899
TITLE: Combination PFC-PWM integrated circuit converter controller - comprises error amplifier which detects intermediate regulated output voltage by sensing current in power factor correction circuit and regulated output voltage of voltage control loop.
DERWENT CLASS: U24
INVENTOR(S): CHEE, A; **HSU, C**; **HWANG, J H**; YU, D
PATENT ASSIGNEE(S): (MICR-N) MICRO LINEAR CORP
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5798635	A	19980825	(199841)*		20

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 5798635	A	CIP of	US 1996-670181	19960620
			US 1997-796128	19970206

PRIORITY APPLN. INFO: US 1997-796128 19970206; US 1996-670181 19960620
AN 1998-480507 [41] WPIDS
CR 1998-260648 [23]
AB US 5798635 A UPAB: 19981014
The controller has a power factor correction stage and a pulse width modulation stage. The power factor correction stage has a control loop for forming a regulated output voltage at the output node. The power factor correction stage provides unity power factor, a regulated intermediate output voltage by sensing a current in the power factor correction circuit and output voltage of voltage control loop.

The power factor correction stage includes an error amplifier which detects the intermediate output voltage. The error amplifier comprises a resistor and a current mirror. The resistance is connected to output node of power factor correction stage and output node of error amplifier.

ADVANTAGE - Provides integrated circuit package having few pins so that controller cost is minimized. Prevents component failure by sensing intermediate regulated output voltage in voltage control loop and DC supply voltage. Uses layer current sources for reliable control of delay time.
Dwg.3a/7

L31 ANSWER 12 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:659781 CAPLUS
DOCUMENT NUMBER: 129:316813
TITLE: Thermal dynamics of side-chain copolymethacrylates as studied by the dielectric spectroscopy and relaxation of second-harmonic generation
AUTHOR(S): Lee, Rong-Ho; Hsiue, Ging-Ho; **Hsu, Che-Kai; Hwang, Jenn-Chiu;** Jeng, Ru-Jong
CORPORATE SOURCE: Department of Chemical Engineering, National Tsing Hua University, Hsinchu, 300, Taiwan
SOURCE: Polymer (1998), 39(26), 6911-6920
CODEN: POLMAG; ISSN: 0032-3861
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of copolymethacrylates with different contents of tolane-based mesogenic groups have been synthesized. The mesogenic group content was characterized with 1H NMR. The phase behaviors were detd. using a differential scanning calorimeter and optical polarizing microscopy. A smectic A phase was obtained when the mesogenic group content was increased up to 80 mol.%. Dielec. relaxation results indicated that the amplitude of the .alpha.-relaxation was suppressed significantly due to the presence of the liq. cryst. phase. The redn. of the mol. motion is beneficial to the enhancement of the temporal stability of the effective second-harmonic coeff. for the polymer with a higher

mesogenic group content. Moreover, the second harmonic coeff. is enhanced as the mesogenic group content increases. The self-alignment nature of the liq. crystal phase is favorable for alignment of the NLO-active mesogenic group under an applied elec. field and preserving such alignment after removal of the elec. field. The relationship between thermal dynamic behavior and second-order nonlinear optical properties is also discussed.

L31 ANSWER 13 OF 34 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:196761 BIOSIS
DOCUMENT NUMBER: PREV199800196761
TITLE: Isodiospyrin, a dual inhibitor of human DNA topoisomerase I and IIa, as a probe to distinguish the relaxation and unknoting reaction of DNA topoisomerase IIa.
AUTHOR(S): **Ting, C.-Y. (1)**; Su, J.-S.; **Hsu, C.-T.**; Chen, T.-Y.; Kuo, Y.-H.; Whang-Peng, J.; **Hwang, J.**
CORPORATE SOURCE: (1) Inst. Mol. Biol., Acad. Sinaca, Taipai Taiwan
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1998) Vol. 39, pp. 424-425.
Meeting Info.: 89th Annual Meeting of the American Association for Cancer Research New Orleans, Louisiana, USA March 28-April 1, 1998 American Association for Cancer Research
. ISSN: 0197-016X.
DOCUMENT TYPE: Conference
LANGUAGE: English

L31 ANSWER 14 OF 34 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:135174 BIOSIS
DOCUMENT NUMBER: PREV199900135174
TITLE: Development of a DNA delivery system using Pseudomonas exotoxin A and a DNA binding region of human DNA topoisomerase I.
AUTHOR(S): **Hwang, Jaulang**; Chen, Tzong-Yueh; **Hsu, Chia-Tse**; Chang, Kai-Hsin; **Ting, Chun-Yuan**; Su, Jin-Shan
CORPORATE SOURCE: Inst. Mol. Biol., Academia Sinica, Nankang, Taipei Taiwan
SOURCE: Cancer Gene Therapy, (Nov.-Dec., 1998) Vol. 5, No. 6 CONF. SUPPL., pp. S9.
Meeting Info.: Seventh International Conference on Gene Therapy of Cancer San Diego, California, USA November 19-21, 1998
ISSN: 0929-1903.
DOCUMENT TYPE: Conference
LANGUAGE: English

L31 ANSWER 15 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1997:269618 CAPLUS
DOCUMENT NUMBER: 126:313163
TITLE: Identification of mutations at DNA topoisomerase I responsible for camptothecin resistance
AUTHOR(S): Wang, Leng-Fang; **Ting, Chun-Yuan**; Lo, Cheng-Kai; Su, Jin-Shan; Mickley, Lyn A.; Fojo, Antonio T.; Whang-Peng, Jacqueline; **Hwang,**

Jaulang
CORPORATE SOURCE: Institute of Molecular Biology, Taipei, 11529, Taiwan
SOURCE: Cancer Res. (1997), 57(8), 1516-1522
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A camptothecin-resistant cell line that exhibits more than 600-fold resistance to camptothecin, designated CPTR-2000, was established from mutagen-treated A2780 ovarian cancer cells. CPTR-2000 cells also exhibit 3-fold resistance to a DNA minor groove-binding ligand Ho33342, a different class of mammalian DNA topoisomerase I inhibitors. However, CPTR-2000 cells exhibit no cross-resistance toward drugs such as Adriamycin, amsacrine, vinblastine, and 4'-dimethyl-epipodophyllotoxin. The mRNA, protein levels, and enzyme-specific activity of DNA topoisomerase I are relatively the same in parental and CPTR-2000 cells. However, unlike the DNA topoisomerase I activity of parental cells, which can be inhibited by camptothecin, that of CPTR-2000 cells cannot. In addn., parental cells after camptothecin treatment results in a decrease in the level of DNA topoisomerase I, whereas CPTR-2000 cells are insensitive to camptothecin treatment. These results suggested that the mechanism of camptothecin resistance is most likely due to a DNA topoisomerase I structural mutation. This notion is supported by DNA sequencing results confirming that DNA topoisomerase I of CPTR-2000 is mutated at amino acid residues Gly717 to Val and Thr729 to IIe. We also used the yeast system to examine the mutation(s) responsible for camptothecin resistance. Our results show that each single amino acid change results in partial resistance, and the double mutation gives a synergetic effect on camptothecin resistance. Because both mutation sites are near the catalytic active center, this observation raises the possibility that camptothecin may act at the vicinity of the catalytic active site of the enzyme-camptothecin-DNA complex.

L31 ANSWER 16 OF 34 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1997:231746 BIOSIS
DOCUMENT NUMBER: PREV199799530949
TITLE: Using the **pseudomonas exotoxin A** as a vehicle to deliver recombinant p53 **protein** into lung cancer cells and enhance their chemosensitivity.
AUTHOR(S): Lai, S.-L. (1); Liao, C.-W.; Lee, H.-H.; Whang-Peng, J.; **Hwang, J.**
CORPORATE SOURCE: (1) Chest Dep., Veterans Gen. Hosp., Taipei 11217 Taiwan
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (1997) Vol. 38, No. 0, pp. 230.
Meeting Info.: Eighty-eighth Annual Meeting of the American Association for Cancer Research San Diego, California, USA April 12-16, 1997
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L31 ANSWER 17 OF 34 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE

10
ACCESSION NUMBER: 96354121 EMBASE
DOCUMENT NUMBER: 1996354121
TITLE: Characterization of monoclonal antibody B7, which neutralizes the cytotoxicity of **Pseudomonas** aeruginosa **exotoxin A**.
AUTHOR: Shang H.-F.; Yeh M.-L.; Lin C.-P.; **Hwang J.**
CORPORATE SOURCE: Institute of Molecular Biology, Academia Sinica,Nankang, Taipei 21529, Taiwan,Province of China
SOURCE: Clinical and Diagnostic Laboratory Immunology, (1996) 3/6 (727-732).
ISSN: 1071-412X CODEN: CDIMEN
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
052 Toxicology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A nontoxic **Pseudomonas** aeruginosa **exotoxin A** (PE), which has the carboxyl-terminal 38 amino acid residues of native PE deleted, was used as an antigen to immunize BALB/c mice, which were then challenged with native PE in order to raise monoclonal antibodies (MAbs) that can neutralize PE cytotoxicity. A murine MAb against PE, designated MAb B7, was established. MAb B7 was characterized in terms of its ability to neutralize PE cytotoxicity, epitope mapping, inhibition of PE receptor binding, and influence on cellular processing of PE and ADP-ribosylation activities. We found that MAb B7 could neutralize PE cytotoxicity in cell culture and in BALB/c mice. The epitope recognized by MAb B7 was mapped to the carboxyl-terminal amino acid residues 575 to 595 of PE. Consistent with the results of epitope mapping, MAb B7 did not block PE receptor-binding activity or the cellular processing of PE but strongly inhibited the ADP-ribosylating activity of PE. In addition, MAb B7 retained strong binding to PE even at pH 4.0, indicating that the complex of MAb B7 and PE is stable in the phagolysosome. On the basis of these observations, the neutralization of PE cytotoxicity by MAb B7 could be due to its binding to the carboxyl terminus of PE. As a result, MAb B7 may interfere with the interaction of the carboxyl-end amino acid residues REDLK of PE with cellular factors. However, we could not rule out the possibility that MAb B7 directly blocks the ADP-ribosylation activity of PE in the cytosol.

L31 ANSWER 18 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 11
ACCESSION NUMBER: 1996:552888 CAPLUS
DOCUMENT NUMBER: 125:187674
TITLE: ADP-ribosylating bacterial toxins: **Pseudomonas exotoxin A**
AUTHOR(S): Chen, Tso Ling; Lin, Lee Chung; **Hwang, Jaulang**; Lin, Chia Po
CORPORATE SOURCE: Natl. Labs. Foods and Drugs, Dep. Health, Taipei, Taiwan
SOURCE: Yaowu Shipin Fenxi (1996), 4(2), 107-114
CODEN: YSFEEP; ISSN: 1021-9498
DOCUMENT TYPE: Journal; General Review

LANGUAGE: Chinese

AB A review and discussion with 31 refs. It is well known that a no. of toxins produced by bacteria exert their action by ADP-ribosylating reaction to certain **proteins** which are essential for normal eukaryotic cellular functions. Most of these toxins are composed of two moieties, A and B. The B moiety mediates the binding to the specific receptor on the surface of toxin-sensitive cells, while the A moiety is responsible for the enzymic ADP-ribosylating activity. **Pseudomonas exotoxin A** (PEA) is the most toxic component of the extracellular products produced by **Pseudomonas** aeruginosa. The three domain model of PEA has been well established: domain I, domain II, and domain III exerting binding, translocation, and ADP-ribosylating activities, resp. Because of the cytotoxic ADP-ribosylating nature of PEA, it has been suggested as a good candidate in the prepn. of immunotoxins. In this minireview article, the authors discuss the structure and function of the bacterial ADP-ribosylating toxins including PEA and compare the differences particularly between PEA and other toxins.

L31 ANSWER 19 OF 34 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 95358835 MEDLINE
DOCUMENT NUMBER: 95358835 PubMed ID: 7632400
TITLE: A target-specific chimeric toxin composed of epidermal growth factor and **Pseudomonas exotoxin A** with a deletion in its toxin-binding domain.
AUTHOR: Liao C W; Hseu T H; **Hwang J**
CORPORATE SOURCE: Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1995 Jul) 43 (3) 498-507.
Journal code: AMC; 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 20000303
Entered Medline: 19950914

AB We have fused the epidermal growth factor (EGF) to the amino terminus of **Pseudomonas exotoxin A** (PE) to create a cytotoxic agent, designated EGF-PE, which preferentially kills EGF-receptor-bearing cells. In this study, we analyzed the effect of the Ia domain, the binding domain of PE on the cytotoxicity of EGF-PE towards EGF-receptor-bearing cells and tried to develop a more potent EGF-receptor-targeting toxin. EGF-PE molecules with sequential deletions at the amino terminus of PE were constructed and expressed in E. coli strain BL21(DE3). The cytotoxicity of these chimeric toxins was then examined. Our results show that the amino-terminal and carboxy-terminal regions of the Ia domain of PE are important for the cytotoxicity of a PE-based targeting toxin. To design a more potent PE-based EGF-receptor-targeting toxin, a chimeric toxin, named EGF-PE(delta 34-220), which had most of the Ia domain deleted but retained amino acid residues 1-33 and 221-252 of this domain, was constructed. EGF-PE(delta 34-220) has EGF-receptor-binding activity but does not

show PE-receptor-binding activity and is mildly cytotoxic to EGF-receptor-deficient NR6 cells. As expected, EGF-PE(delta 34-220) is a more potent cytotoxic agent towards EGF-receptor-bearing cells than EGF-PE(delta 1-252), where the entire Ia domain of PE was deleted. In addition, EGF-PE(delta 34-220) was shown to be extremely cytotoxic to EGF-receptor-bearing cancer cells, such as A431, CE81T/VGH, and KB-3-1 cells. We also found that EGF-PE(delta 34-220) was highly expressed in BL21(DE3) and could be easily purified by urea extraction. Thus, EGF-PE(delta 34-220) can be a useful cytotoxic agent towards EGF-receptor-bearing cells.

L31 ANSWER 20 OF 34 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 95337870 MEDLINE
DOCUMENT NUMBER: 95337870 PubMed ID: 7613234
TITLE: Serum thyrotropin-binding inhibiting immunoglobulin and thyroperoxidase antibody in Graves' hyperthyroidism after 131I therapy.
AUTHOR: **Hsu C H**; Lee L S; Chang J J; Liao S T; Chen S M; **Hwang J Y**; Lo N I
CORPORATE SOURCE: Department of Nuclear Medicine and Clinical Pathology, Taipei Municipal Jen-Ai Hospital, Taiwan, R.O.C.
SOURCE: JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1995 Jan-Feb) 94 (1-2) 5-9.
Journal code: BLQ; 9214933. ISSN: 0929-6646.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
(CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950822

AB Seventeen patients who received radioiodine (131I) therapy for Graves' hyperthyroidism had serial blood samples taken before therapy and after therapy for a period of at least 1 year. At 1 year post-therapy, six patients were hypothyroid. Seven patients were euthyroid, and four patients were hyperthyroid. Prior to isotope administration, 14 patients had detectable serum thyrotropin-binding inhibiting immunoglobulin (TBII) and 16 patients had detectable serum thyroperoxidase antibody (TPOAb). Three to 6 months after therapy, 11 of 14 TBII-positive patients demonstrated a marked increase (> 10%) in serum TBII activity. Four patients out of 11 developed hypothyroidism and six of the 11 developed euthyroidism. A decrease in TBII was observed in three patients who developed hyperthyroidism. In the three patients with undetectable TBII prior to therapy, two had high titers of TPOAb. Seven patients demonstrated a marked increase in TPOAb 3 to 6 months after therapy. Of these, four developed hypothyroidism and three developed euthyroidism, whereas three of the four patients who had a marked decrease in TPOAb developed hyperthyroidism. This study demonstrated that an increase in serum TBII and TPOAb activity 3 to 6 months after 131I therapy, may be useful in predicting which patients may develop euthyroidism or hypothyroidism after 1 year of 131I therapy.

L31 ANSWER 21 OF 34 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 94339754 MEDLINE

DOCUMENT NUMBER: 94339754 PubMed ID: 7914775
TITLE: Hormonal change in an adult with Prader-Willi syndrome: report of a case.
AUTHOR: Shiah C J; Lee L S; **Hwang J Y**; Liao S T; **Hsu C H**; Lin W Y
CORPORATE SOURCE: Department of Internal Medicine, Taipei Municipal Jen-Ai Hospital, Taiwan R.O.C.
SOURCE: JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1994 Apr) 93 (4) 324-7.
Journal code: BLQ; 9214933. ISSN: 0929-6646.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 19941005
Entered Medline: 19940920

AB We report a classical case of Prader-Willi syndrome (PWS) in an adult with typical interstitial deletion of chromosome 15, and emphasize the study of hormonal change. This 21-year-old female had PWS face characteristics, small hands and feet, marked obesity, mental retardation, growth retardation, absence of puberty and amenorrhea. She also had the characteristic history of infantile hypotonia, poor feeding, failure to thrive and then improved appetite, followed by obesity from the age of four years. She had compulsive hyperphagia, to the extent of stealing and lying to take food. Chromosome study with high resolution banding technique revealed a small interstitial deletion at band q12 of chromosome 15, which is characteristically found in a majority of patients with PWS. Hormonal study revealed hypogonadism and growth hormone deficiency of supposed hypothalamic origin. She also had non-insulin-dependent diabetes mellitus with decreased pancreatic insulin reserve.

L31 ANSWER 22 OF 34 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1996:344703 BIOSIS
DOCUMENT NUMBER: PREV199699067059
TITLE: Engineering of **Pseudomonas exotoxin A** into useful **proteins** for disease treatment.
AUTHOR(S): **Hwang, Jaulang**
CORPORATE SOURCE: Inst. Mol. Biol., Acad. Sinica, Taipei Taiwan
SOURCE: Journal of the Chinese Biochemical Society, (1994) Vol. 23, No. 2, pp. 173-174.
Meeting Info.: Symposium Honoring C. C. Yang Taipei, Taiwan July 15, 1994
ISSN: 0379-7368.
DOCUMENT TYPE: Conference
LANGUAGE: English

L31 ANSWER 23 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 15
ACCESSION NUMBER: 1996:302627 CAPLUS
DOCUMENT NUMBER: 124:333996
TITLE: Engineering of **Pseudomonas exotoxin A** into useful **proteins** for disease treatment
AUTHOR(S): Hseuh, Kuan-Hua; Shang, Huey-Fang; Wang,

Leng-Fang; Lo, Cheng-Kai; Liao, Chao-Wei; **Hwang, Jaulang**
CORPORATE SOURCE: Institute Molecular Biology, Academia Sinica, Taipei, 11529, Taiwan
SOURCE: J. Chin. Biochem. Soc. (1994), 23(2), 135-151
CODEN: JCBSB5; ISSN: 0379-7368
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 80 refs. **Pseudomonas exotoxin A** (PE) is one of the most toxic components of the extracellular products produced by **Pseudomonas** aeruginosa. PE is a single chain **polypeptide** with three structural domains. In order to execute PE toxicity, PE should contain at least three functional domains, namely binding to cells, translocation across a membrane, and ADP-ribosylation of elongation factor 2 (EF-2). Recent studies have correlated the structural domains of PE with specific biol. functions. The domains responsible for receptor-binding, translocation, and ADP-ribosylation were found to correspond to structural domain Ia (residues 1-252), domain II (residues 253-364), and domain III (residues 405-613), resp. Based on the known functional property of each PE domain, the possibility of engineering **Pseudomonas exotoxin A** (PE) into useful **proteins** for disease treatment is evaluated. The goal is to test the possibility of a reality. The following projects are discussed: (1) engineering of PE into a tumor-specific toxin for cancer therapy; (2) engineering of PE into a tumor suppressor **protein** for cancer therapy; (3) engineering of PE into an antiviral vaccine; and (4) engineering of PE into a vaccine against Pseudomonas infection.

L31 ANSWER 24 OF 34 TOXLINE
ACCESSION NUMBER: 1994:84255 TOXLINE
DOCUMENT NUMBER: BIOSIS-94-24281
TITLE: FUNCTIONAL ANALYSIS OF IA DOMAIN OF **PSEUDOMONAS EXOTOXIN A.**
AUTHOR: **HWANG J**; LIAO C-W; HSEU T-H
SOURCE: (1994). Vol. 8, No. 7 A1463. 85TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, WASHINGTON, D.C., USA, MAY 21-25, 1994. FASEB JOURNAL.
CODEN: FAJOEC.
FILE SEGMENT: BIOSIS
LANGUAGE: English
ENTRY MONTH: 199409
AB BIOSIS COPYRIGHT: BIOL ABS. RRM MEETING ABSTRACT PSEUDOMONAS MAMMAL EPIDERMAL GROWTH FACTOR CYTOTOXICITY

L31 ANSWER 25 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 16
ACCESSION NUMBER: 1993:509765 CAPLUS
DOCUMENT NUMBER: 119:109765
TITLE: An EGF-**Pseudomonas exotoxin A** recombinant **protein** with a deletion in toxin binding domain specifically kills EGF receptor bearing cells
AUTHOR(S): Lee, Chi Hon; Lee, E Ching; Tsai, Shih Tzer; Kung, Hsing Jien; Liu, Yin Chang; **Hwang, Jaulang**
CORPORATE SOURCE: Inst. Mol. Biol., Acad. Sin., Taipei, Taiwan

SOURCE: Protein Eng. (1993), 6(4), 433-40
CODEN: PRENE9; ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors constructed two chimeric toxins; one composed of EGF and **pseudomonas exotoxin A** (PE), designated EGF-PE and the other composed of EGF and PE with a deletion of the Ia domain (cell-binding domain), designated EGF-PE(.DELTA.Ia). Both chimeric toxins reacted with anti-EGF and anti-PE antibodies. The cell-killing expts. showed that EGF-PE, but not EGF-PE(.DELTA.Ia), was cytotoxic to the murine fibroblast cell line NR6, which carried the PE receptor, but not the EGF receptor. However, after NR6 was transfected with DNA for the expression of human EGF receptor, the transfected cell line, designated NRHER5, overexpressed human EGF receptors and became sensitive to EGF-PE(.DELTA.IA). The cytotoxicity of EGF-PE(.DELTA.Ia), but not EGF-PE, to NRHER5 can be completely blocked by an excess amt. of EGF. To completely reverse the cytotoxicity of EGF-PE on NRHER5, both the EGF receptor pathway and the PE receptor pathway need to be blocked. These results suggest that EGF-PE exhibits both EGF and PE binding activities, while EGF-PE(.DELTA.IA) possesses only EGF binding activity. Thus, EGF-PE(.DELTA.Ia) may be a better chimeric toxin than EGF-PE in terms of target specificity to EGF receptor bearing cells. The authors therefore examd. the cytotoxicity of EGF-PE(.DELTA.Ia) to various human cancer cell lines. Human cancer cells contg. more EGF receptors are more sensitive to EGF-PE(.DELTA.Ia).

L31 ANSWER 26 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 17

ACCESSION NUMBER: 1992:626462 CAPLUS
DOCUMENT NUMBER: 117:226462

TITLE: **Pseudomonas exotoxin A**-EGF mutant chimeric **protein** as an indicator for identifying amino acid residues important in EGF-receptor interaction

AUTHOR(S): Shiah, Her Shyong; Chen, Tzong Yueh; Chang, Chi Ming; Chow, Judy T.; Kung, Hsing Jien; **Hwang, Jaulang**
CORPORATE SOURCE: Inst. Mol. Biol., Acad. Sin., Taipei, 11529, Taiwan

SOURCE: J. Biol. Chem. (1992), 267(33), 24034-40
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Epidermal growth factor (EGF) was fused to the carboxyl end of a modified **pseudomonas exotoxin A** that has its toxin binding domain deleted. This chimeric toxin designated as PE(.DELTA.Ia)-EGF kills A431 cells through the EGF receptor-mediated pathway. In this study, a random mutagenesis approach was used to make point mutations on EGF, followed by replacing the wild type EGF in PE(.DELTA.Ia)-EGF with these EGF mutants. Fourteen different PE(.DELTA.Ia)-EGFmutants were constructed, and their EGF receptor binding activity as well as their cytotoxicity to A431 cells were examd. Results showed that individual mutations of Val19 to Glu and Val34 to Asp in the EGF domain of PE(.DELTA.Ia)-EGFmutants resulted in an increase in the binding affinity to EGF receptor and cytotoxicity to A431 cells. On the other hand, individual mutations of His16 to Asp and Gly18 to Ala in the EGF domain of PE(.DELTA.Ia)-EGFmutants led to a decrease

in the binding affinity to EGF receptor and cytotoxicity to A431 cells. In addn., mutations of any of the cysteine residues of EGF in PE(.DELTA.Ia)-EGFmutants resulted in the loss of their binding activity to EGF receptor and a corresponding loss of their cytotoxicity. Thus, the cytotoxicity of PE(.DELTA.Ia)-EGFmutant to EGF receptor-bearing cells may be used as an indicator to screen mutations of EGF important in EGF-receptor interactions.

L31 ANSWER 27 OF 34 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 90036994 MEDLINE
DOCUMENT NUMBER: 90036994 PubMed ID: 2553721
TITLE: Identification of the carboxyl-terminal amino acids important for the ADP-ribosylation activity of **Pseudomonas exotoxin A**.
AUTHOR: Chow J T; Chen M S; Wu H C; **Hwang J**
CORPORATE SOURCE: Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Republic of China.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Nov 5) 264 (31) 18818-23.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198911
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19891128

AB The ADP-ribosylation domain of **Pseudomonas exotoxin A** (PE) has been identified to reside in structural domain III (residues 405-613) and a portion of domain Ib (residues 385-404) of the molecule (Hwang, J., FitzGerald, D. J., Adhya, S., and Pastan, I. (1987) Cell 48, 129-136). To further determine the carboxyl end region essential for ADP-ribosylation activity, we constructed sequential deletions at the carboxyl-terminal of PE. Our results show that a clone with a deletion of the carboxyl-terminal amino acid residues from Arg-609 to Lys-613 and replaced with Arg-Asn retained wild-type PE ADP-ribosylation activity. Deletion of the terminal amino acid residues from Ala-596 to Lys-613 and replaced with Val-Ile-Asn reduced ADP-ribosylation activity by 75%, while deletions of 36 or more amino acids from the carboxyl terminus completely lose their ADP-ribosylation activity. These modified PEs were also examined for their ability to block PE cytotoxicity. Our results shown that modified PEs which lost their ADP-ribosylation activity correspondingly lost their cytotoxicity. Furthermore, extracts containing PE fragments without ADP-ribosylation activity were able to block the cytotoxic activity of intact PE. Our results thus indicate that carboxyl-terminal amino acids in the Ser-595 region are crucial for ADP-ribosylation activity and, consequently, cytotoxicity of PE. The modified PEs which have lost their ADP-ribosylation activity may also be a route to new PE vaccines.

L31 ANSWER 28 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 19
ACCESSION NUMBER: 1989:133248 CAPLUS
DOCUMENT NUMBER: 110:133248
TITLE: Structure and function relationship of **Pseudomonas exotoxin A**

. An immunochemical study
AUTHOR(S): **Hwang, Jaulang**; Chen, Mei Shya
CORPORATE SOURCE: Inst. Mol. Biol., Acad. Sin., Taipei, Taiwan
SOURCE: J. Biol. Chem. (1989), 264(4), 2379-84
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Antisera were raised against **Pseudomonas exotoxin A** (PE) and domains Ia and III to study the structure-function relationships of PE. Anti-PE antibody (AbPE) abolished the ADP-ribosylation activity of PE. However, neither antidomain Ia antibody nor antidomain III antibody inhibited the ADP-ribosylation activity of PE. This suggests that the inhibition of ADP-ribosylation by AbPE results from the binding of AbPE to the region between domains Ia and III. Since the binding of AbPE did not inhibit NAD hydrolysis in the absence of elongation factor 2, the inhibitory effect of AbPE on ADP-ribosylation may be due to steric hindrance rather than a direct action on the catalytic function. Thus, the interface between domain Ia and III may be the site of entry of elongation factor 2 during ADP-ribosylation. Either AbPE or antidomain Ia antibody, but not antidomain III antibody, was able to reverse the inhibition of **protein** synthesis by PE and to block its cytotoxicity. Rabbits immunized with domain Ia acquired tolerance against 100 .mu.g of PE injected s.c. These results suggest that domain Ia is the cell-binding domain of PE and may be used for vaccination against PE-mediated diseases.

L31 ANSWER 29 OF 34 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 90257560 MEDLINE
DOCUMENT NUMBER: 90257560 PubMed ID: 2517634
TITLE: Apolipoproteins A-I and B in non-insulin-dependent diabetes mellitus.
AUTHOR: Lee L S; **Hwang J Y**; Chang J J; **Hsu C H**; Liao S T; Lo I L
SOURCE: TAIWAN I HSUEH HUI TSA CHIH JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1989 Nov-Dec) 88 (11-12) 1139-42.
Journal code: I6V; 0413761. ISSN: 0371-7682.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900720
Last Updated on STN: 19900720
Entered Medline: 19900628

AB In recent years apolipoproteins A-I and B examinations have been performed on patients with coronary artery disease as a better predictor of the severity of atherosclerosis. In the present study, 21 treated male and 22 treated female patients with non-insulin-dependent diabetes mellitus (NIDDM) were examined and compared with controls of the same sex, age and body mass (23 males, 21 females). Cholesterol, triglyceride, LDL-cholesterol in male and female patients with NIDDM were significantly higher than in male and female controls. HDL-cholesterol in male and female patients with NIDDM was not different from those of male and female controls. Apolipoproteins A-I and B in male and female patients with NIDDM

were higher than in male and female controls. [Apolipoproteins A-I (g/L) male 1.40 +/- 0.21 vs 1.25 +/- 0.15, p less than 0.005; female 1.56 +/- 0.23 vs 1.42 +/- 0.24, p less than 0.025. Apolipoproteins B (g/L) male 1.29 +/- 0.30 vs 0.97 +/- 0.22, p less than 0.001; female 1.34 +/- 0.34 vs 0.98 +/- 0.35, p less than 0.001.] Discrepancy between the higher apolipoprotein A-I and the normal HDL-cholesterol in in NIDDM supports the theory of altered composition of HDL particles in diabetic patients. The controversy between the higher apolipoprotein A-I and the higher incidence of atherosclerosis in patients with NIDDM makes the clinical usefulness of this laboratory measurement doubtful in these patients.

L31 ANSWER 30 OF 34 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 87310337 MEDLINE
DOCUMENT NUMBER: 87310337 PubMed ID: 3625163
TITLE: Left pyriform sinus fistula complicated by acute suppurative thyroiditis: report of a case.
AUTHOR: Liao S T; Lee L S; **Hsu C H; Hwang J Y**; Chiang T P; Chou T J; Siauw C P; Chen P H
SOURCE: TAIWAN I HSUEH HUI TSA CHIH JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1987 May) 86 (5) 569-72. Journal code: I6V; 0413761. ISSN: 0371-7682.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19871008

L31 ANSWER 31 OF 34 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 22
ACCESSION NUMBER: 1988:50200 CAPLUS
DOCUMENT NUMBER: 108:50200
TITLE: Functional domains of Pseudomonas exotoxin identified by deletion analysis of the gene expressed in E. coli
AUTHOR(S): **Hwang, Jaulang**; Fitzgerald, David J.; Adhya, Sankar; Pastan, Ira
CORPORATE SOURCE: Div. Cancer Biol. Diagn., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Cell (Cambridge, Mass.) (1987), 48(1), 129-36
CODEN: CELLB5; ISSN: 0092-8674
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Pseudomonas Exotoxin A** (PE) is a single chain toxin with 3 structural domains that inhibits **protein** synthesis in eukaryotic cells by catalyzing ADP ribosylation of elongation factor 2. To study the function of these domains, different portions of the PE structural gene were deleted and these constructs were expressed in Escherichia coli using an inducible T7 promoter. These studies indicate that structural domain Ia is required for cell recognition, that structural domain II is required to translocate the toxin across a cellular membrane, and that structural domain III and a portion of domain Ib are required for ADP ribosylation activity. Toxin lacking domain La is about 100-fold less toxic to mice than is intact PE and should be a useful mol. for the construction of immunotoxins.

L31 ANSWER 32 OF 34 MEDLINE
ACCESSION NUMBER: 88033267 MEDLINE
DOCUMENT NUMBER: 88033267 PubMed ID: 2444605
TITLE: Mutant KB cells with decreased EGF receptor expression: biochemical characterization.
AUTHOR: **Hwang J**; Richert N; Pastan I; Gottesman M M
CORPORATE SOURCE: Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892.
SOURCE: JOURNAL OF CELLULAR PHYSIOLOGY, (1987 Oct) 133 (1) 127-34.
Journal code: HNB; 0050222. ISSN: 0021-9541.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198712
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 20000303
Entered Medline: 19871217

AB Mutants of the human KB carcinoma cell line resistant to a cytotoxic conjugate of epidermal growth factor and Pseudomonas exotoxin (EGF-PE) express a pleiotropic phenotype, which includes reduced levels of 125I-EGF binding, without altered affinity for EGF (Lyall et al., 1987). Here, the EGF-toxin (ET) resistant mutants were further characterized with respect to the amount and size of the EGF receptor and the level of EGF receptor RNA. These data indicate that decreased binding of 125I-EGF in the mutants is due to reduced amounts of EGF receptor, which is associated with decreased mRNA levels. Changes in other **proteins** in the ET mutants were also examined. Five of the six ET mutants had a decrease in a 78,000 Mr- membrane glycoprotein. In addition, an increase in a **protein** with a Mr- of 40,000 and a pl = 8.0 was found in all the mutants, and an increase in a series of **proteins** with a Mr- of 36,000 and a pl of 6.3-6.5 was found in some of the mutants. These results confirm the pleiotropic nature of the EGF-PE resistant mutants and show that reduced EGF binding is due to altered expression of the EGF receptor gene in the mutants.

L31 ANSWER 33 OF 34 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 86226110 MEDLINE
DOCUMENT NUMBER: 86226110 PubMed ID: 3869637
TITLE: Serum thyroid hormones in thyroid and nonthyroid disorders: with special emphasis on reverse triiodothyronine measurement.
AUTHOR: **Hsu C H**; Lee L S; **Hwang J Y**
SOURCE: TAIWAN I HSUEH HUI TSA CHIH JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1985 Dec) 84 (12) 1313-22.
Journal code: I6V; 0413761. ISSN: 0371-7682.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198607
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860710

L31 ANSWER 34 OF 34 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 86113985 MEDLINE
DOCUMENT NUMBER: 86113985 PubMed ID: 3866832
TITLE: Serum gastrin in upper gastro-intestinal disorders.
AUTHOR: **Hwang J Y; Hsu C H;** Chen P H; Lee L S; Wang C S; Siauw C P
SOURCE: TAIWAN I HSUEH HUI TSA CHIH JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, (1985 Oct) 84 (10) 1159-64. Journal code: I6V; 0413761. ISSN: 0371-7682.
PUB. COUNTRY: TAIWAN: Taiwan, Province of China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198603
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860307

FILE 'HOME' ENTERED AT 15:24:15 ON 13 NOV 2001

- key terms disclose
** May contain prev. viewed citations

FILE 'REGISTRY' ENTERED AT 15:36:17 ON 14 NOV 2001
L1 70 S (GONADOTROPIN RELEASING HORMONE ? OR "GONADOTROPIN-RELE
L2 1 S 9034-40-6/RN
L3 71 S L1 OR L2

FILE 'CAPLUS' ENTERED AT 15:36:34 ON 14 NOV 2001
L1 70 SEA FILE=REGISTRY ABB=ON PLU=ON (GONADOTROPIN RELEASING HORMONE ? OR "GONADOTROPIN-RELEASING HORMONE" ?)/CN
L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON 9034-40-6/RN
L3 71 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2
L4 746 SEA FILE=CAPLUS ABB=ON PLU=ON PSEUDOMONAS(S)((EXOTOXIN OR EXO TOXIN)(W)A)
L5 501 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (L3 OR PEPTIDE OR POLYPEPTIDE OR POLYPROTEIN OR PROTEIN OR GNRH OR (GN OR GONADOTROPIN)(W)(RELEAS? HORMON? OR RH) OR VACCINIA)
L6 3 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND (MULTIPLE(3A)COPIES)

L6 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:525737 CAPLUS
DOCUMENT NUMBER: 133:236494
TITLE: Vaccination against **gonadotropin-releasing hormone** (**GnRH**) using toxin receptor-binding domain-conjugated **GnRH** repeats
AUTHOR(S): Hsu, Chia-Tse; Ting, Chun-Yuan; Ting, Chun-Jen; Chen, Tzong-Yueh; Lin, Chia-Po; Whang-Peng, Jacqueline; Hwang, Jaulang
CORPORATE SOURCE: Graduate Institute of Life Science, National Defense Medical Center, Institute of Molecular Biology, Academia Sinica, Taipei, 11529, Taiwan
SOURCE: Cancer Res. (2000), 60(14), 3701-3705
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A method for the prepn. of an immunogen contg. **multiple copies** of a self-**peptide** in linear alignment was designed to overcome the difficulty of inducing an immune response to poorly immunogenic **peptide** antigens. DNA fragments encoding multiple repeats of the self-**peptide** were generated by a new technique, termed template-repeated polymerase chain reaction (TR-PCR), which could be subcloned into an expression vector for prodn. of **peptide** repeats as an immunogen. This approach was tested by constructing fusion **proteins** contg. the receptor-binding domain of **Pseudomonas exotoxin A** and **multiple copies** of the 10-residue sequence of the **peptide** hormone **gonadotropin-releasing hormone** (**GnRH**). Immunization of female rabbits with the immunogen that contained the exotoxin receptor-binding domain and 12 copies of **GnRH** (PEIa-GnRH12) resulted in the generation of high-titer antibodies specific for **GnRH**. Although at equal molar basis of the **GnRH** moiety, the immunogen that contained single copy of **GnRH** (PEIa-GnRH1) induced low-titer anti-**GnRH** antibodies. These observations suggest that the presence of multiple **peptide** repeats is a key factor in eliciting an immune response. In addn., anti-**GnRH**

antibodies effectively neutralized **GnRH** activity in vivo, as demonstrated by the degeneration of the ovaries in the injected rabbits. Because anti-**GnRH** antibody could be functionally analogous to **GnRH** antagonist, which has been used to treat patients with ovarian cancer, vaccination of PEIa-GnRH12 presents a potential therapeutic application for the treatment of **GnRH** -sensitive ovarian cancer.

IT **9034-40-6**, LH-RH
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(vaccination against **GnRH** multimer-toxin fusion construct induces neutralizing antibodies to)

REFERENCE COUNT: 16
REFERENCE(S):
(1) Baselga, J; Cancer Res 1998, V58, P2825 CAPLUS
(2) Baselga, J; J Clin Oncol 1996, V14, P737 CAPLUS
(3) Baselga, J; J Natl Cancer Inst 1993, V85, P1327 CAPLUS
(4) Conn, P; Fed Proc 1984, V43, P2351 CAPLUS
(5) Eidne, K; Science (Washington DC) 1985, V229, P989 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:464633 CAPLUS
DOCUMENT NUMBER: 119:64633
TITLE: Coordinate regulation of siderophore and **exotoxin A** production: Molecular cloning and sequencing of the **Pseudomonas** aeruginosa fur gene
AUTHOR(S): Prince, Robert W.; Cox, Charles D.; Vasil, Michael L.
CORPORATE SOURCE: Health Sci. Cent., Univ. Colorado, Denver, CO, 80262, USA
SOURCE: J. Bacteriol. (1993), 175(9), 2589-98
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 5.9-kb DNA fragment was cloned from P. aeruginosa PA103 by its ability to functionally complement a fur mutation in Escherichia coli. A fur null mutant E. coli strain that contains **multiple copies** of the 5.9-kb DNA fragment produces a 15-kDa **protein** which cross-reacts with a polyclonal anti-E. coli Fur serum. Sequencing of a subclone of the 5.9-kb DNA fragment identified an open reading frame predicted to encode a **protein** 53% identical to E. coli Fur and 49% identical to Vibrio cholerae Fur and Yersinia pestis Fur. Although there is extensive homol. among these Fur **proteins**, Fur from P. aeruginosa differs markedly at its C-terminus from all of the other Fur **proteins**. It has been proposed that this region is a metal-binding domain in E. coli Fur. A pos. selection procedure involving the isolation of Mn-resistant mutants was used to isolate mutants of strain PA103 that produce altered Fur **proteins**. These Mn-resistant Fur mutants constitutively produce siderophores and exotoxin A when grown in concns. of normally repress their prodn. A multicopy plasmid carrying t aeruginosa fur gene restores Mn susceptibility and wild-type

regulation of exotoxin A and siderophore prodn. in these Fur mutants.

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:52559 CAPLUS
DOCUMENT NUMBER: 116:52559
TITLE: Regulation of toxA and regA by the Escherichia coli fur gene and identification of a fur homolog in Pseudomonas aeruginosa PA103 and PA01
AUTHOR(S): Prince, R. W.; Storey, D. G.; Vasil, A. I.; Vasil, M. L.
CORPORATE SOURCE: Health Sci. Cent., Univ. Colorado, Denver, CO, 80262, USA
SOURCE: Mol. Microbiol. (1991), 5(11), 2823-31
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A multicopy plasmid contg. the E. coli fur gene was introduced into P. aeruginosa strain PA103C. This strain contains a toxA-lacZ fusion integrated into its chromosome at the toxA locus. .beta.-Galactosidase synthesis in this strain is regulated by iron, as is seen for exotoxin A prodn. Beta-galactosidase synthesis and exotoxin A prodn. in PA103C contg. **multiple copies** of E. coli fur was still represented in low iron conditions. The transcription of regA, a pos. regulator of toxA, was also found to be inhibited by **multiple copies** of the E. coli fur gene. In addn., the ability of PA103C contg. **multiple copies** of E. coli fur to produce protease was greatly reduced relative to PA103C contg. a vector control. A polyclonal rabbit serum contg. antibodies that recognize E. coli Fur was used to screen whole-cell exts. from Vibrio cholerae, Shigella flexneri, Salmonella typhimurium, and P. aeruginosa. All strains tested expressed a **protein** that was specifically recognized by the anti-Fur serum. These results suggest that Fur structure and function are conserved in a variety of distinct bacterial genera and that at least some of these different genera use this regulatory **protein** to control genes encoding virulence factors.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXLIT, TOXLINE, PHIC, PHIN' ENTERED AT 15:39:20 ON 14 NOV 2001)
L7 16 S L6
L8 6 DUP REM L7 (10 DUPLICATES REMOVED)

L8 ANSWER 1 OF 6 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-309780 [33] WPIDS
DOC. NO. CPI: C2001-095841
TITLE: New **polypeptides** having **multiple copies** of a **peptide** antigen fused to the receptor binding domain of a Pseudomonas exotoxin, useful as a vaccine and for generating antibodies for diagnostic and/or therapeutic procedures.
DERWENT CLASS: B04 D16
INVENTOR(S): HSU, C; HWANG, J; TING, C
PATENT ASSIGNEE(S): (SINI-N) ACAD SINICA; (SINI-N) ACAD SINICA INC
COUNTRY COUNT: 28
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1090994	A2	20010411	(200133)*	EN	15
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
AU 2000062500	A	20010412	(200133)		
CA 2304377	A1	20010405	(200133)	EN	
NZ 507368	A	20010629	(200140)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1090994	A2	EP 2000-304253	20000519
AU 2000062500	A	AU 2000-62500	20001005
CA 2304377	A1	CA 2000-2304377	20000428
NZ 507368	A	NZ 2000-507368	20001005

PRIORITY APPLN. INFO: US 1999-412558 19991005
AN 2001-309780 [33] WPIDS
AB EP 1090994 A UPAB: 20010615
NOVELTY - A new **polypeptide** comprises a receptor binding domain of a **Pseudomonas exotoxin A** or its functional variant; and at least two copies of a **peptide** sequence.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid (N1) encoding the **polypeptide**;

(2) a method of producing the **polypeptide**; and

(3) a vaccine composition comprising at least one **polypeptide** or at least nucleic acid cited above, and optionally a pharmaceutical carrier.

ACTIVITY - Immunostimulant.

Mice and pig were immunized with PEIa-**GnRH** 12 (a PEIa plasmid expressing 12 repeats of **gonadotropin releasing hormone** (**GnRH**)). The mice received a 100 mu l bolus containing 10 mu g PEIa-**GnRH** 12 and 12 mu g aluminum phosphate for each injection. In addition, a 24 day-old pig was injected once with a 1 ml bolus containing 10 mg PEIa-**GnRH** 12 and 250 mu g aluminum phosphate. **GnRH** -specific antibodies were readily elicited in the mice and pig, indicating that the antigens can elicit an immune response in a variety of animals.

MECHANISM OF ACTION - Vaccine.

USE - The **polypeptide** is useful as a vaccine. The **polypeptide** is useful for generating antibodies that specifically bind a monomeric **peptide** sequence. Such antibodies are useful in diagnostic and/or therapeutic procedures that require the enhancement, inhibition or detection of any molecule that contains the epitope presented by the **peptide** sequence.

Dwg.0/3

L8 ANSWER 2 OF 6 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000402342 MEDLINE
DOCUMENT NUMBER: 20374289 PubMed ID: 10919636
TITLE: Vaccination against **gonadotropin-**

releasing hormone (GnRH)
using toxin receptor-binding domain-conjugated
GnRH repeats.
AUTHOR: Hsu C T; Ting C Y; Ting C J; Chen T Y; Lin C P; Whang-Peng J; Hwang J
CORPORATE SOURCE: Graduate Institute of Life Science, National Defense Medical Center, Academia Sinica, Taipei, Taiwan.
SOURCE: CANCER RESEARCH, (2000 Jul 15) 60 (14) 3701-5. Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000824

AB A method for the preparation of an immunogen containing multiple copies of a self-peptide in linear alignment was designed in order to overcome the difficulty of inducing an immune response to poorly immunogenic peptide antigens. DNA fragments encoding multiple repeats of the self-peptide were generated by a new technique, termed template-repeated polymerase chain reaction (TR-PCR), which could be subcloned into an expression vector for production of peptide repeats as an immunogen. This approach was tested by constructing fusion proteins containing the receptor-binding domain of Pseudomonas exotoxin A and multiple copies of the 10-residue sequence of the peptide hormone gonadotropin-releasing hormone (GnRH). Immunization of female rabbits with the immunogen that contained the exotoxin receptor-binding domain and 12 copies of GnRH (PEIa-GnRH12) resulted in the generation of high-titer antibodies specific for GnRH. Although at equal molar basis of the GnRH moiety, the immunogen that contained single copy of GnRH (PEIa-GnRH1) induced low-titer anti-GnRH antibodies. These observations suggest that the presence of multiple peptide repeats is a key factor in eliciting an immune response. In addition, anti-GnRH antibodies effectively neutralized GnRH activity in vivo, as demonstrated by the degeneration of the ovaries in the injected rabbits. Because anti-GnRH antibody could be functionally analogous to GnRH antagonist, which has been used to treat patients with ovarian cancer, vaccination of PEIa-GnRH12 presents a potential therapeutic application for the treatment of GnRH -sensitive ovarian cancer.

L8 ANSWER 3 OF 6 MEDLINE
ACCESSION NUMBER: 2000161470 MEDLINE
DOCUMENT NUMBER: 20161470 PubMed ID: 10696480
TITLE: Expression of ptxR and its effect on toxA and regA expression during the growth cycle of Pseudomonas aeruginosa strain PAO1.
AUTHOR: Colmer J A; Hamood A N
CORPORATE SOURCE: Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock 79430, USA.

CONTRACT NUMBER: AI-33386 (NIAID)
SOURCE: CANADIAN JOURNAL OF MICROBIOLOGY, (1999 Dec) 45 (12) 1008-16.
Journal code: CJ3; 0372707. ISSN: 0008-4166.
PUB. COUNTRY: Canada
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000407
Last Updated on STN: 20000407
Entered Medline: 20000327

AB The expression of the toxA and regA genes in **Pseudomonas** aeruginosa is negatively regulated by iron at the transcriptional level. We have previously described ptxR, an **exotoxin A** regulatory gene which appears to enhance toxA expression through regA. In this study, we have tried to determine if ptxR expression correlates with its effect on toxA and regA expression throughout the growth cycle of P. aeruginosa strain PAO1. This was done using Northern blot hybridization experiments (with toxA, regA, and ptxR probes), and ptxR transcriptional fusion studies. To avoid problems related to the presence of **multiple copies** of ptxR in PAO1, we have constructed a PAO1 strain (PAO1-XR) that carries only two ptxR genes in its chromosome. Our results showed that when PAO1-XR was grown in iron-limited conditions, the increase in **exotoxin A** activity and the accumulation of toxA mRNA appeared at about mid- to late-exponential phase. A similar increase in the accumulation of regA mRNA was detected. Both regA transcripts, T1 and T2, were enhanced in PAO1-XR. In iron-sufficient medium, neither toxA nor regA mRNA was detected at any time point in the growth cycle of PAO1-XR. In contrast, the accumulation of ptxR mRNA was detected throughout the growth cycle of PAO1-XR under both iron-deficient and iron-sufficient conditions. The presence of iron in the growth medium also had no effect on the level of beta-galactosidase activity produced by a ptxR-lacZ fusion in PAO1. These results suggest that (i) the enhancement in toxA expression by ptxR correlates with the enhancement in regA expression; (ii) ptxR affects the expression of the regA P1 and P2 promoters; (iii) ptxR expression precedes its effect on toxA and regA expression; and (iv) unlike toxA and regA, the overall expression of ptxR throughout the growth cycle of PAO1 is not negatively regulated by iron.

L8 ANSWER 4 OF 6 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 93239684 MEDLINE
DOCUMENT NUMBER: 93239684 PubMed ID: 8478325
TITLE: Coordinate regulation of siderophore and **exotoxin A** production: molecular cloning and sequencing of the **Pseudomonas** aeruginosa fur gene.
AUTHOR: Prince R W; Cox C D; Vasil M L
CORPORATE SOURCE: Department of Microbiology/Immunology, University of Colorado Health Sciences Center, Denver 80262.
CONTRACT NUMBER: AI15940 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 May) 175 (9) 2589-98.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L00604
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930611
Last Updated on STN: 19970203
Entered Medline: 19930526

AB A 5.9-kb DNA fragment was cloned from **Pseudomonas** aeruginosa PA103 by its ability to functionally complement a fur mutation in Escherichia coli. A fur null mutant E. coli strain that contains **multiple copies** of the 5.9-kb DNA fragment produces a 15-kDa **protein** which cross-reacts with a polyclonal anti-E. coli Fur serum. Sequencing of a subclone of the 5.9-kb DNA fragment identified an open reading frame predicted to encode a **protein** 53% identical to E. coli Fur and 49% identical to Vibrio cholerae Fur and Yersinia pestis Fur. While there is extensive homology among these Fur **proteins**, Fur from P. aeruginosa differs markedly at its carboxy terminus from all of the other Fur **proteins**. It has been proposed that this region is a metal-binding domain in E. coli Fur. A positive selection procedure involving the isolation of manganese-resistant mutants was used to isolate mutants of strain PA103 that produce altered Fur **proteins**. These manganese-resistant Fur mutants constitutively produce siderophores and **exotoxin A** when grown in concentrations of iron that normally repress their production. A multicopy plasmid carrying the P. aeruginosa fur gene restores manganese susceptibility and wild-type regulation of **exotoxin A** and siderophore production in these Fur mutants.

L8 ANSWER 5 OF 6 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 93202713 MEDLINE
DOCUMENT NUMBER: 93202713 PubMed ID: 8454322
TITLE: LasR of **Pseudomonas** aeruginosa is a transcriptional activator of the alkaline protease gene (apr) and an enhancer of **exotoxin A** expression.
AUTHOR: Gambello M J; Kaye S; Iglewski B H
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, New York 14642.
CONTRACT NUMBER: AI33713 (NIAID)
T32GM07356 (NIGMS)
SOURCE: INFECTION AND IMMUNITY, (1993 Apr) 61 (4) 1180-4.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930507
Last Updated on STN: 20000303
Entered Medline: 19930422

AB The lasR gene of **Pseudomonas** aeruginosa is required for transcription of the genes for elastase (lasB) and LasA protease (lasA), two proteases associated with virulence. We report here that the alkaline protease gene (apr) also requires the lasR gene for transcription. Alkaline protease mRNA was absent in the lasR mutant

PAO-R1 and present when an intact lasR gene was supplied in trans as determined by Northern (RNA) analysis. The lasR gene also enhances **exotoxin A** production. **Exotoxin A** activity in supernatants of PAO-R1 were 30% less than in supernatants of the parental strain, PAO-SR. **Multiple copies** of lasR in trans in PAO-R1 in increased toxin A activity to twice the parental levels. Analysis of PAO-R1 containing the toxA promoter fused to beta-galactosidase suggests that LasR acts at the toxA promoter or at upstream toxA mRNA sequences. beta-Galactosidase activity was approximately 40% lower in PAO-R1 than in the parental strain, PAO-SR. Furthermore, the effect of LasR on the toxA promoter is not due to the stimulation of transcription of regA, a transcriptional activator of toxA. No difference in chloramphenicol acetyltransferase (CAT) activity was noted between PAO-SR and PAO-R1 containing transcriptional regA promoter-CAT gene fusions. These results broaden the regulatory dominion of lasR and suggest that the lasR gene plays a global role in P. aeruginosa pathogenesis.

L8 ANSWER 6 OF 6 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 92140047 MEDLINE
DOCUMENT NUMBER: 92140047 PubMed ID: 1779768
TITLE: Regulation of toxA and regA by the Escherichia coli fur gene and identification of a Fur homologue in Pseudomonas aeruginosa PA103 and PA01.
AUTHOR: Prince R W; Storey D G; Vasil A I; Vasil M L
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver 80262.
CONTRACT NUMBER: AI15940 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1991 Nov) 5 (11) 2823-31. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920329
Last Updated on STN: 19970203
Entered Medline: 19920310

AB A multicopy plasmid containing the Escherichia coli fur gene was introduced into **Pseudomonas** aeruginosa strain PA103C. This strain contains a toxA-lacZ fusion integrated into its chromosome at the toxA locus. Beta-galactosidase synthesis in this strain is regulated by iron, as is seen for **exotoxin A** production. Beta-galactosidase synthesis and **exotoxin A** production in PA103C containing **multiple copies** of E. coli fur was still repressed in low iron conditions. The transcription of regA, a positive regulator of toxA, was also found to be inhibited by **multiple copies** of the E. coli fur gene. In addition, the ability of PA103C containing **multiple copies** of E. coli fur to produce protease was greatly reduced relative to PA103C containing a vector control. A polyclonal rabbit serum containing antibodies that recognize E. coli Fur was used to screen whole-cell extracts from Vibrio cholerae, Shigella flexneri, Salmonella typhimurium and **Pseudomonas** aeruginosa. All strains tested expressed a **protein** that was specifically recognized by the anti-Fur serum. These results and those described above suggest that Fur

structure and function are conserved in a variety of distinct bacterial genera and that at least some of these different genera use this regulatory **protein** to control genes encoding virulence factors.

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